acta

physiologica

latino

americana

VOLUMEN IX
Nº 1

1959

ACTA PHYSIOLOGICA LATINOAMERICANA

Editada por la Asociación Ciencia e Investigación

COMITES EDITORIALES

Miembros Honorarios

Bernardo A. Houssay (Argentina)
Carlos Chagas (Brasil)
Paulo Eneas Galvão (Brasil)
Eduardo Cruz Coke (Chile)
Alejandro Lipschutz (Chile)
Carlos Monge (Perú)
Thales Martins (Brasil)

SECRETARIO DE REDACCION
Dr. Manuel R. Malinow

ADMINISTRADORA
Srta. Josefina Yanguas

Obligado 2490, Buenos Aires — Argentina

Suscripción

Cada tomo (cuatro números trimestrales) $ 250.— m/n. Exterior: 7.50 dólares

REPRESENTANTES:

BRASIL: Editora Guanabara, Weissmann-Koogan, Ltda.
Rua do Ouvidor, 132, Río de Janeiro.

EE. UU.: Stechert - Hafner Inc.
31 East 10th Street, New York 3, N. Y.

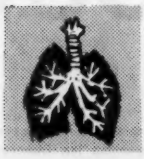
Squibb ofrece

KENACORT

...por primera vez todos los efectos beneficiosos del tratamiento corticosteroide sin retención de sodio y agua o euforia artificial

PARA SUS PACIENTES CON:

asma bronquial
alergia



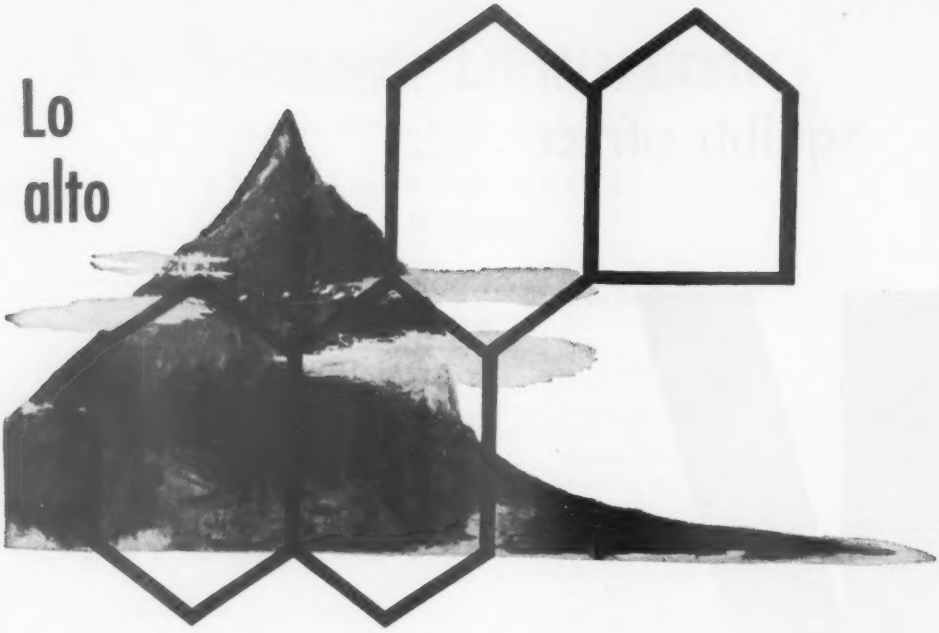
artritis

dermatosis

TRIAMCINOLONA SQUIBB

**el de mayor efectividad
con mínimo de efectos secundarios
y el más amplio margen de seguridad.**

Lo alto



y lo *bajo* de

Ledercort*

Triamcinolona

...un nuevo corticosteroide creado por LEDERLE para reducir a su mínimo los inconvenientes con que se ha tropezado hasta ahora con la terapia esteroidea.-

Lo alto - un nuevo **NIVEL ELEVADISIMO** de efectos antiinflamatorios a dosis más bajas (como término medio un tercio menos que las de prednisona o prednisolona).

Lo bajo - un nuevo **NIVEL BAJISIMO** de efectos hormonales colaterales como los observados por todos los corticosteroides anteriores: • No hay retención de sodio No hay pérdida de potasio • No se altera el equilibrio psiquico • Menos incidencia de úlcera péptica y ósteoporosis

Indicado en: • Artritis reumatoidea • Desórdenes alérgicos, incluso asma bronquial • Dermatosis • Muchos otros estados en los que los corticosteroides han resultado provechosos.

Envases: Frascos de 10 y 20 tabletas ranuradas de 2 y 4 mg.

Lederle

*Marca Registrado

DIVISION LABORATORIOS LEDERLE
Cyanamid de Argentina S. A.
Charcas 5051 - Buenos Aires

CYANAMID

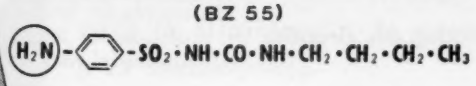
SANDOZ ARGENTINA S. A. I. C.

2

Antidiabéticos orales "BOEHRINGER"

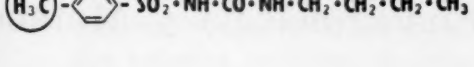
NADISAN

(BZ 55)



ARTOSIN

(D 860)



Esidrex

(Hidroclorotiazida)

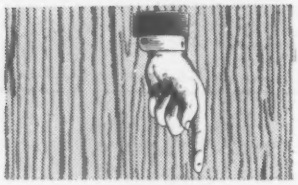
Sali-diurético
para la eliminación de edemas
y el tratamiento
de la hipertensión

El Esidrex es un diurético nuevo no mercurial, que actúa por vía oral, eliminador de cloruro de sodio, singularmente bien tolerado, efectivo a bajas dosis y de acción constante.

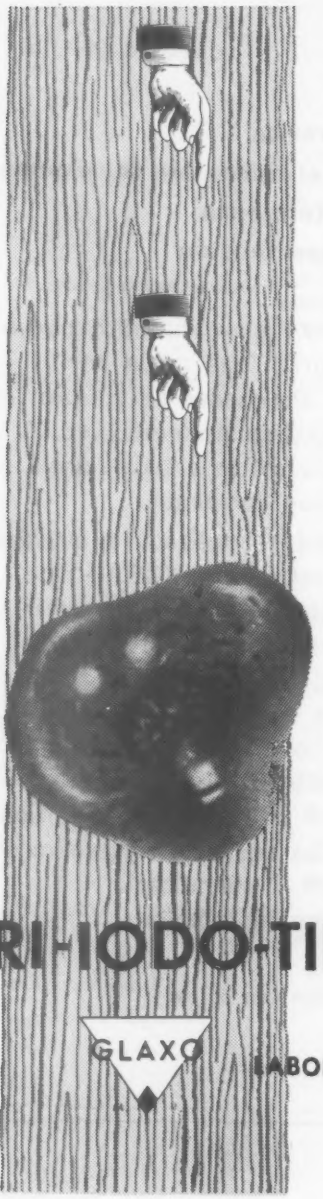
El Esidrex —sintetizado e investigado en los Laboratorios CIBA— es igualmente un antihipertensivo que refuerza la acción de otros hipotensores, insertándose útilmente en el esquema terapéutico clásico, completando el tratamiento de base con Serpasol® o Adelfán®, permitiendo reducir las dosis de Apresolina® o Ecolid® y liberalizar el régimen hiposódico

Comprimidos con 50 mg

CIBA



estímulo metabólico directo



La L-triiodotironina es probablemente la etapa final de la hormona tiroactiva.

— De acción directa en los procesos metabólicos celulares.
— Constituye un tratamiento seguro y fácilmente controlable de los **estados hipometabólicos** y de la obesidad, dismenórreas, insuficiencia metabólica de la vejez, etc.

Posología: 20 a 60 microgramos repartidos en dos o tres tomas diarias.

Presentación: frasco con 50 comprimidos ranurados (cada comprimido contiene 20 microgramos de L-triiodotironina sódica).

TRI-IODO-TIRONINA GLAXO

GLAXO

LABORATORIOS GLAXO (Argentina) S. A. C. e I

DISEL

en

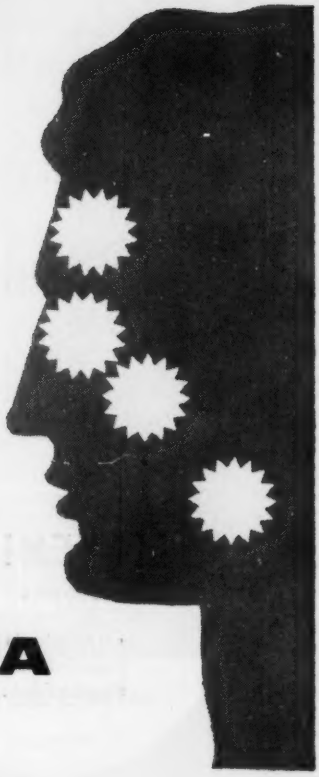
Rinitis agudas

Sinusitis - Faringitis

y en

Rinitis alérgicas

o inflamatorias



DISEL

con

HIDROCORTISONA

Los rinofaringeos más completos para uso de niños y adultos.

- **No producen irritación.**
- **No interfieren el movimiento ciliar.**
- **Actividad mucolítica elevada.**
- **Hidrocortisona *EN SOLUCION.***

Ambos productos también preparados para uso infantil

PRESENTACION: Envases - pulverizadores de 10 cm^3.

LABORATORIOS ANDRÓMACO

Av. Ing. Huergo 1139-51 Buenos Aires

Medicamentos Roche para la terapéutica anticoagulante

Tratamiento

LIQUEMINE
Roche

preparado de heparina pura standardizada

MARCOUMAR
Roche

derivado cumarínico por vía oral, de acción difusa uniforme

Inactivación

PROTAMINA
Roche

neutralizante de la hemorragia por sobredosificación de la heparina, para uso intravenoso

KONAKION
Roche

vitamina K_1, inactivadora de los efectos de los derivados cumarínicos

24 escalones que pueden hospitalizarlo

Un esfuerzo tan común como subir un tramo de escaleras, confronta al enfermo de angina péctoris con la angustiosa pregunta: "¿Lo resistirá mi corazón?".

El esfuerzo provoca el ataque... y el miedo a los ataques va gradualmente limitando las actividades del paciente. Al cabo de un tiempo, hasta los intervalos entre ataques tienen poca semblanza de vida normal.

Suprima el factor "miedo". En cada 4 de 5 pacientes, la profilaxis con Peritrate reduce la frecuencia e intensidad de los ataques anginósos, mejora los trazados electrocardiográficos y aumenta la tolerancia al ejercicio.

Una nueva sensación de libertad se apodera del "inválido cardíaco", restituyéndole la confianza en sí mismo al sentirse útil y partícipe del mundo que le rodea, aunque no debe olvidar que le siguen prohibidos los ejercicios activos.

La profilaxia con Peritrate es simple: basta administrar una tableta de 10 ó 20 mg. *antes de las comidas* y al acostarse.

Presentación:

Peritrate 10 mg. - frascos de 50 y 100 tabletas.
Peritrate 20 mg. - frascos de 50 tabletas
Peritrate (10 mg.) con Fenobarbital (15 mg.) - frascos de 50 tabletas.
Peritrate (10 mg.) con Aminofilina (100 mg.) - frascos de 50 tabletas.

Peritrate

Tetranitrato de Pentaeritritol

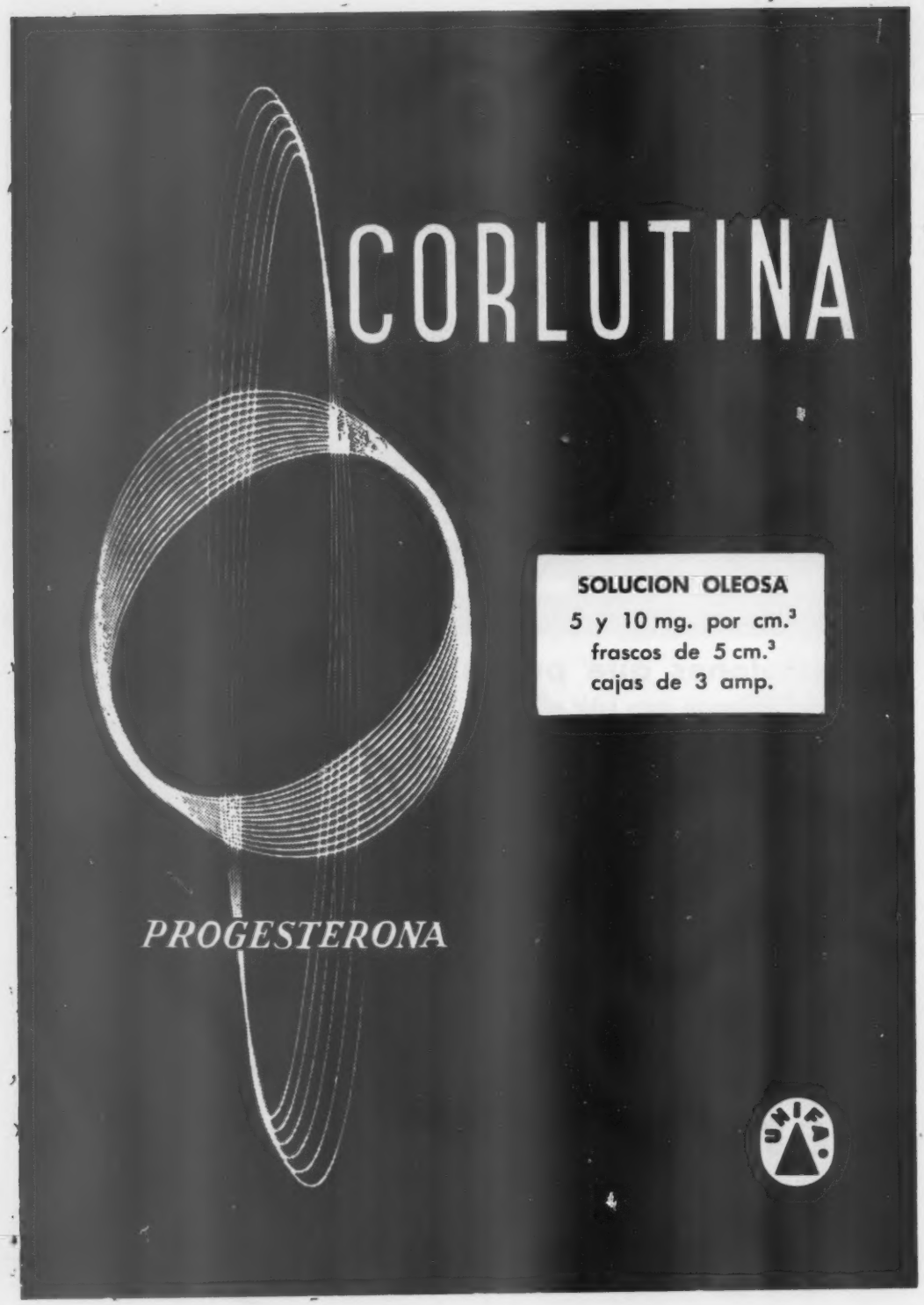
Muestras y Literatura:

WILLIAM R. WARNER & CIA., S. A.
Sarmiento 3401 Buenos Aires

WARNER-CHILCOTT
Laboratories DIV. MORRIS PLAINS, N.J., U.S.A.

MAS DE 100 AÑOS AL SERVICIO DE LA PROFESION MEDICA

P4-58


CORLUTINA
SOLUCION OLEOSA
5 y 10 mg. por cm.³
frascos de 5 cm.³
cajas de 3 amp.
PROGESTERONA
UNIA

iniciamos

una nueva dimensión en terapéutica córticosteroide!

La gran era inicial de los córticosteroides se abrió con la introducción de Cortone (Cortisona). Hoy Merck Sharp & Dohme exhibe orgullosamente la culminación de la primera década - con DECADRON (dexametasona) - un descubrimiento, que señala un nuevo orden de magnitudes en terapéutica córticosteroide.

Este es

Decadron

DEXAMETASONA

para tratar más pacientes con mayor eficacia

Envases de 10 y 30 tabletas

M S D

MERCK SHARP & DOHME

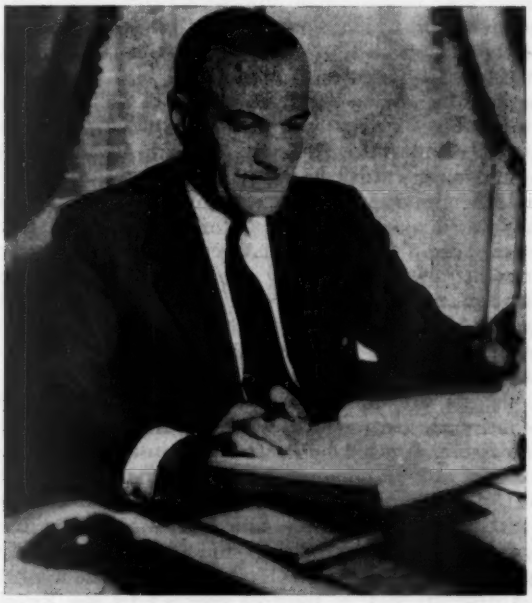
es sinónimo de investigación

Eduardo Braun Menéndez

1903 - 1959

THE death of Eduardo Braun Menéndez, which occurred in an airplane accident on the day of his fifty-sixth birthday, has meant for the world of science and Argentine intellectual life the loss of one of their outstanding exponents, his students have lost an eminent teacher and his colleagues a friend who was a man of rare and noble spirit.

From his earliest years Dr. Braun Menéndez showed his vocation for science.



The research work he did while still a student was the basis for his MD thesis on "The influence of the diencephalon and the hypophysis on arterial blood pressure", for which he was awarded the Buenos Aires Faculty of Medicine Prize in 1934. From then onwards without interruption till his death he continued his work in research, and was thus able to make important contributions to the knowledge of the functions of the circulatory system in normal

and pathological conditions. He followed a precise and strict experimental method and resorted to no less precise clinical observation when it was necessary for the elucidation of a given problem.

Later he studied the heart sounds and together with Dr. Oscar Orías, another eminent argentine physiologist prematurely deceased, collected the results they had obtained on this subjct in a book "The heart sounds in normal and pathological conditions", which appeared in the Spanish edition in 1937 and shortly afterwards in an English translation. The exactness with which the facts were stated and the originality and soundness of the conclusions have made it a classic. The book was awarded one of the yearly prizes given for scientific work by the Argentine Government.

The cardiac rhythm and heart metabolism were subjects to which Dr. Braun Menéndez contributed interesting work, part of which was begun in the Department of Physiology of University College, London, under Professor Lovatt Evans in 1938.

The following year, while he formed one of a team with Drs. J. C. Fasciolo, L. F. Leloir, J. M. Muñoz and A. C. Taquini, a series of remarkable investigations were carried out to elucidate the mechanism of arterial hypertension of renal origin. The results were published in numerous papers and collected in a book "Renal hypertension" of which Spanish, English and Italian editions were published. This book was also awarded one of the prizes for scientific work given by the Argentine Government.

Renal function and salt and water metabolism in their relation to hypertension were the subject of many interesting studies. Dr. Braun Menéndez contributed several chapters to Houssay's Human Physiology, amongst them those on renal function on which he was a recognized authority.

Dr. Braun Menéndez was an outstanding figure in the University. Shortly after he was appointed associate professor of Physiology in the Buenos Aires University Medical School in 1943, the Argentine Universities were the object of an outrageous act of aggression by the National Government, several of the most eminent of their professors were dismissed, amongst them Professor B. A. Houssay. Dr. Braun Menéndez, who was one of Professor Houssay's associates, resigned then and again in 1946 when the outrage was repeated after a transitory amendment with reposition of the dismissed professors. He could not become an accomplice of those determined to destroy academic freedom. He continued his research work in the Institute of Biology and Experimental Medicine, directed by Professor Houssay, which he, together with other colleagues were able to organize thanks to voluntary contributions.

During the following years both in Argentine and abroad, Universities and learned societies sought him out, and his teaching and the result of his researches were heard in Uruguay, Brazil and Chile, in the United States and Canada, in England, France, Belgium, Holland and Sweden.

When the University had its freedom restored by the Revolution of 1955 he was appointed Professor of Physiology in the Buenos Aires University Medical School. He threw himself into the work of repairing the enormous damage done by a decade of corruption, with all his enthusiasm and power, and although the difficulties he encountered were many and considerable, his optimism never faltered. It is a grievous misfortune that he should have gone when the task of reconstruction has hardly begun. The Argentine University has lost him who could have been one of its greatest builders.

He had a very clear idea of what a University should be, and of the fundamental reforms necessary for the Argentine Universities to become what they should be. He expressed his ideas with force and brilliance revealing the fervour of an apostle of an intellectual creed. His teachings on the subject have been preserved in many publications which will serve as a guide for those who share his ideals and wish to continue his work. He hoped to see the University dedicated to the search for truth, and free from the utilitarianism which is now predominant. He worked unceasingly to convert it from a narrow professionalism to the love of scientific and humanistic knowledge, because he knew that the desinterested search for truth is not only the proper use of intelligence, but also confers a professional and technical competence not achieved by mere ad hoc learning. The achievement of this aim seems very remote at present, but his struggle will not have been in vain, the coming years will show the solidity of his work and his contribution to the rebuilding of the University will stand out.

He knew how important science is, not only for the material progress and prosperity of humanity but also for its spiritual welfare. The advancement of science, the encouragement of scientific research was, therefore, one of his interests. He was one of the founders, in 1938, of the Argentine Cardiological Society, which he presided in 1951, and was also a founding member of the Argentine Physiological Society in 1953. This same year he founded the Catholic Institute of Science. He contributed to the foundation of the Revista Argentina de Cardiología, which he edited for a time. Ciencia e Investigación was due to his initiative and since its first number appeared in 1945 he had been active on its editorial board. These and ACTA PHYSIOLOGICA LATINOAMERICANA are also one of his creations and he nursed them with constant care.

He made a great contribution to the advancement of scientific work in Argentina through the Argentine Association for the Advancement of Science, of which he was Secretary from 1945 to 1948, and the Argentine Scientific Society which he presided since 1956. Finally when the National Council for Scientific and Technical Research was created he became a member of its governing board and presided its Committee for Medical Sciences.

All these achievements, which gave him an international reputation and many distinctions and honours from scientific institutions and universities, both national and foreign, were the fruits of the activities of an uncommon personality. He was a simple man in that he had none of those dark and twisted corners in the mind which hide one knows not what. On the contrary he put everything of himself into the clear light of day, whith frankness and loyalty so translucent as to appear ingenuous. Nevertheless this simplicity was made up of many different facets; the man of science following a severe and strict method in his work; the understanding and affectionate teacher; the crusader for the upbuilding of the University and the advancement of science, were harmoniously allied to a keen sportsman, a highly cultivated devotee of music and literature, and at a deeper level, a warm friend, a loving husband and father and a christian who lived his faith in everyone of his deeds. Three aspects of his rich personality may be considered outstanding: first, his integrity, all these different facets were harmonized perfectly, so there was no contradiction in his being or in his acts. Secondly, his generosity of spirit, which made him scrupulous in being just and giving everyone his due, knowing in theory and in practice that what is first owed to our neighbours is brotherly love paid in

service, kindly help and sincere affection; and finally his modesty, which was natural and without ostentation, enabling him to receive all the many honours bestowed on him without vanity, as mere accidents which are not the source but only the recognition of the merit which has won them.

Braun Menéndez did not leave this world alone; he was accompanied on that last journey by his daughter Magdalena, his secretary and technician, who like himself was humble, hardworking and lovable.

The death of a man who deserves the epithet of magnanimous, in the sense used by the Greeks, that is to say, endowed with greatness of soul, leaves a sensation of emptiness and loss in those who had the privilege of knowing him. It also leaves in them a sensation of gratitude, because the mere fact of having known him has helped to improve their mind and fortify their virtue, they have been made men. The pupils, colleagues and friends of Eduardo Braun Menéndez owe much to him, they can pay their debt by continuing the work for the good of humanity he had to leave unfinished.

J. T. Lewis.

NUCLEIC ACID CONTENT AND AMYLASE ACTIVITY IN THE PANCREAS OF THE RAT FOLLOWING ADRENALECTOMY AND CORTISONE ADMINISTRATION (*)

A. Sesso and R. Migliorini

(Department of Histology [Professor L. Lison] and Department of Physiology [Professor M. R. Covian]. Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brazil.)

The rôle of the adrenals on the nucleic acid patterns of digestive glands, has been approached only by cytochemical observations. Celular atrophy and decrease in cytoplasmic pentosenucleic acid (PNA) as revealed by basophilic staining were described in the zymogenic cells of fundic glands after the removal of the adrenals (Abrams & Baker, 1952; Baker & Brigman, 1954). A more severe atrophy than that induced by adrenalectomy was found in the gastric serous cells of hypophysectomized animals (Baker & Abrams, 1954).

Hydrocortisone produced a marked recovery in the cytoplasmic basophilic material of gastric chief cells in the hypophysectomized rats, while only a slight effect was obtained by force-feeding or by the administration of somatotrophin (Baker & Abrams, 1955). In view of the lack of quantitative data about the influence of the adrenals upon the desoxyribonucleic acid (DNA) and PNA of digestive glands, it seemed worthwhile to study the nucleic acids levels and the amylase activity of the rat pancreas after adrenalectomy, as well as after replacement therapy by cortisone.

The pancreas can be considered a suitable gland for such studies since its exocrine portion, which represents most of the gland, is constituted only by one type of functional element, the serous cells. Islet tissue, even when increased in weight under the stimulus of hormones, such as cortisone and ACTH, does not represent more than about two per cent of the organ (Kinash & Haist, 1954).

(*) This work was aided by grants from the Conselho Nacional de Pesquisas and the Rockefeller Foundation.

Received for publication, July 30th, 1958.

Methods

Wistar rats, of both sexes and same age were used. The animals in each sex group were divided in three sub-groups: normal unoperated, adrenalectomized and adrenalectomized-cortisone injected. Cortisone was administered twice daily subcutaneously, during ten days, since the day of the operation. The daily dose was 1 mg for each animal. Normal and adrenalectomized rats were injected with saline. All animals had unrestricted access to food. Throughout the experiment the animals were given to drink one per cent sodium chloride. In the day following the last injection all the animals were sacrificed by ether inhalation. The pancreas was immediatly removed, weighed after careful dissection from lymph nodes and adipose tissue, and homogenized with hundred times its weight of cold distilled water. During that procedure, the homogenizer remained immersed in an ice bath. Nucleic acids were estimated by a combined Schneider (17) –Schmidt– Thannhauser procedure (16). After the extraction of the acid-soluble compounds and phospholipids, the residual material was hydrolised from an aliquot of the homogenate in NaOH 0.5 N during 18 hours at 37° C. DNA was precipitated by trichloracetic acid and estimated by its phosphorus content by the method of Pereira (1939) as modified by Rabinovitch (1951). The PNA remaining in the supernatant was determined with orcinol according to Brown (1946).

The colour developed with orcinol was compared to a standard curve obtained with a sample of yeast nucleic acid containing 8.35 per cent of phosphorus. The results of pentosenucleic acid are expressed as the phosphorus content of the standard sample. Amylase activity was assayed by the method of Smith and Roe (1949) in a proper dilution of the one per cent pancreas homogenate with cold distilled water. Amylase results are expressed as Smith and Roe units per 100 mg of the wet weight of pancreas (U.S.R./100 mg).

The nucleic acids concentration is expresed as μg of P per 100 mg of wet weight of pancreas.

Statistical method

The six sets of measurements performed in the normal (N) adrenalectomized (A) and adrenalectomized cortisone (AC) injected rats of both sexes, were submited to the analysis of variance, according to the following scheme: the variance due to experimental error (17 degrees of freedom) was estimated by subtracting the sum of squares (SM) between groups (5 degrees of freedom) from the total sum of squares (22 degrees of freedom); in the male adrenalectomized group, one animal was not successfully operated upon; the missing data has been substituted by an estimate calculated from the mean of the data of the other animals in the group, one degree of freedom being lost in the operation). The sum of squares and the degrees of freedom for the variance between groups has been partitioned in five orthogonal comparisons, each one with one degree of freedom. The table I gives the general scheme of the partition, the values given in this table are the coefficients for the calculations by the orthogonal polynomial coefficients.

In planning the experiments, it has been decided to test the hypothesis that adrenalectomy alters the weight and the patterns of the nucleic acids, whilst the administration of cortisone to the previously adrenalectomized animals

TABLE I

Comparison	Male N	Male AC	Male A	Female N	Female AC	Female A
1. – Normal (N) × adrenalectomized cortisone-injected (AC)	+	—	0	+	—	0
2. – Adrenalectomized (A) versus + adrenalectomized cortisone-injected	+	+	−2	+	+	−2
3. – Males versus Females	+	+	+	—	—	—
4. – Interaction I due to sex	+	—	0	—	+	0
5. – Interaction II due to sex	+	+	−2	+	+	−2

compensate the lack of the adrenals and reestablish the pattern of the normal animals. This led to the first orthogonal comparisons: 1) Control versus cortisone-treated adrenalectomized and 2) Adrenalectomized versus control and cortisone-treated adrenalectomized. A third comparison has been provided for testing the eventual differences due to sex. Since the experiment is organized in a 3×2 factorial design, two comparisons are available for interactions. The meaning of the interactions is the following one. The Interaction I is the eventual discrepancy between sexes in relation to the compensation of adrenalectomy by the administration of cortisone. The interaction II is the eventual discrepancy between sexes in relation to the action of adrenalectomy.

Results

The experimental results are summarized in Table II.

To abbreviate the presentation of the statistical analysis in Table III are given only the mean squares (MS) and the F ratios obtained from the analysis of variance made on the original data.

From the analysis of the table III, the following conclusions may be drawn:

1) *Pancreas weight.* – Adrenalectomy induces a highly significant decrease in the both the absolute and the relative weight of the pancreas (comparisons II A and II B with respectively $F = 30.2$ and $F = 20.8$). This result answers to the main problem studied in this paper, but the analysis of variance displays interesting side-lights on various other problems. No significant differences appear to exist between the absolute pancreas weight in male and in female of the same age (comparison III A, $F = 2.87$). However since the body weight of the female is less than the weight of male same aged, the relative pancreas weight is higher in the female and the difference is statistically significant (comparison III B, $F = 33.0$). On the other hand the administration of cortisone to the adrenalectomized animals compensate the effect of the adrenalectomy in relation to the absolute weight of the pancreas (comparison I.A., $F = 0.61$) but

TABLE II

	MALE			FEMALE		
	Normal	Adrenalectomized cortisone injected	Adrenalectomized	Normal	Adrenalectomized cortisone injected	Adrenalectomized
Nº of rats	4	4	4 +	4	4	4
Init. B. Wt. (gm.)	172 ± 6.0 (*)	169 ± 6.9	171 ± 4.6	139 ± 5.9	139 ± 4.9	136 ± 3.9
Final B. Wt. (gm.)	204 ± 1.2	158 ± 6.3	152 ± 4.0	160 ± 5.8	133 ± 9.2	142 ± 6.9
Mean pancreas Wt. mg.	686 ± 40.8 (**)	742	450	720	728	600
Mean pancreas Wt. mg per 100 g body Wt. g	336 ± 26.0	467	294	449	593	422
DNA-P μg/100 mg pancreas	40.3 ± 2.12	38.8	54.2	40.5	37.0	44.4
PNA-P μg/100 mg pancreas	180.7 ± 6.40	180.7	152.7	197.0	188.7	157.5
DNA-P total μg per pancreas ...	276 ± 16.9	296	240	307	269	266
Amylase-Smith and Roe-units/100 mg pancreas	2697 ± 383.1	3647	787	3337	3800	1522

(*) Standard Error $= \frac{SD}{\sqrt{n}}$

(**) Standard Error estimated from the residual error of the analysis of variance.

is unable to compensate the loss of body weight (Table I) with the consequence that the relative pancreas weight increases significantly in the cortisone-treated adrenalectomized animals (comparison I, B, $F = 27.6$).

2) *Desoxyribonucleic Acid (DNA).* – The analysis of variance of the DNA μg P/100 mg wet weight data reveals a significant type II interaction (comparison VC, $F = 6.02$). Thus, the main effects of the adrenalectomy tested by the comparison IIC, and of the sex, tested by the comparison IIIC, need to be examined in order to lead to a correct interpretation of the facts.

The examination of the original data (Table II) shows that adrenalectomy increases the pancreatic DNA concentration in both sexes but that this increase is much greater in the male than in the female: in the male, the mean increase

TABLE III

Means squares and F values from the analysis of variance performed on the data presented in Table I

	A Pancreas weight, mg		B Pancreas Wt. body Wt. 100 mg/100 g		C Deoxyribonucleic phosphorus μg/100 mg		D Ribonucleic phosphorus μg/100 mg		E Total deoxyribonucleic phosphorus/ per pancreas		F Amylase		Degrees of freedom
	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	
I. – Normal X cortisone-injected adrenalectomized	4096	0.61	75213	27.6(**)	25	1.39	68	0.43	306	0.28	1995100	3.40	1
II. – Adrenalectomized X normal + Adrenalectomized cortisone-injected	200984	30.2(**)	56650	20.8(**)	550.8	30.7(**)	5355	32.6(**)	6302	5.83(*)	26181300	44.6(**)	1
III. – Males X Females	19153	2.87	89913	33.0(**)	87.9	4.89(*)	561	3.42	280	0.26	1555500	2.65	1
IV. – Interaction I due to sex ...	2401	0.36	150	0.05	3.8	0.21	68	0.41	3481	3.22	237600	1	1
V. – Interaction II due to sex ..	26227	3.93	93	0.03	108	6.02(*)	72	0.44	690	0.63	153000	1	1
IV. – Experimental Error	6660		2725		17.94		163.9		1081		587100		17

(*) Significant at the probability level of 0.05. F 0.05 for 1 and 17 D.F = 4.45.
(**) Significant at the probability level of 0.01. F 0.01 for 1 and 17 D.F = 8.40.

may be estimated by $54.2 - (40.3 + 38.8) / 2 = 14.6$ μg/100 mg whilst in the female it is $44.4 - (40.5 + 37.0) / 2 = 5.6$ μg/100 mg.

If t 0.05 is the 0.05 t value of the Student distribution at the 0.05 significance level, s2 the error mean square, n_1 and n_2 the number of items in two experimental groups, the lowest significant difference between the means of groups is

$$d_{0.05} = t_{0.05} \sqrt{s^2 \frac{n_1 + n_2}{n_1 \; n_2}} = 2.11 \sqrt{17.94 \; \frac{12}{32}} = 5.48$$

Thus it appears that the increase in the DNA concentration observed after adrenalectomy is just above the limit of the 0.05 level in the female (5.6), while it is about 2.66 times this value in the male (14.6). This fact accounts for the significant type II interaction. The interpretation of the significant value for the sex comparison IIIC ($F = 4.89$) must be made cautiously due to the significant interaction. Examination of the original data shows that in the normal or in the cortisone-treated adrenalectomized animals, there is no sex difference since the estimate of the difference is $(40.3 + 38.8) - (40.5 + 37.0) = 1.6$ μg/100 mg, much inferior to the lowest significant difference, equals 4.47. In the adrenalectomized groups the DNA concentration in the male exceeds that of the female by $54.2 - 44.4 = 9.8$ μg/100 gr, a significant value since it is about 1.5 times the lowest significant difference, equals 6.33.

The analysis of total DNA data shows a difference between adrenalectomized animals and the two other experimental groups (comparison II E, $F = 5.83$), while no difference is observed between normal and adrenalectomized cortisone-injected rats (comparison I E, $F = 0.28$).

3) *PNA and amylase.* – As it may be seen in table III, PNA and amylase concentration were significantly lowered in the adrenalectomized animals (comparison II D and II F; with $F = 32.6$ and $F = 44.6$ respectively). No difference can be observed between normal and adrenalectomized cortisone-injected rats in regards with PNA and amylase concentration (comparison ID and IF with respectively $F = 0.43$ and $F = 3.40$).

Discussion

The pancreas diminishes in weight when the pituitary gland is removed (Koster, 1930; Bryans et al, 1952). Acinar cells undergo a severe cytoplasmic atrophy, as shown by cytological examination and by the increase in DNA concentration (Sesso et al, 1955). The concentration of cytoplasmic elements as PNA, amylase and proteinase, decreases (Sesso, et al, 1955; Barret, Nishikawara & Haist, 1955; Baker, Reid & Thoms, 1956) (Kinash & Haist, 1955; Kinash & Haist, 1955). When somatrophin and thyroid powder were administered to hypophysectomized rats, it was observed that both increased the pancreas weight; however the orally administrated thyroid powder proved to be most effective in restoring the amylase activity (Nishikawara et al, 1954). Although thyroid seems to exert a considerable influence upon exocrine pancreas activity in the hypophysectomized rat, it may be suspected that the hypophysis acts on the pancreas, at least partially, through the adrenal cortex. This was suggested by the fact that cortisone and ACTH administration into hypophysectomized rats increased the pancreas weight (Kinash & Haist, 1954; Baker & Abrams, 1955). Our present

results not only support the above supposition, but also stress the importance of the adrenals on exocrine pancreas activity.

The results herein reported on the concentration of the nucleic acids and amylase activity after adrenalectomy are similar to those already observed in the pancreas of the hypophysectomized rat (Sesso et al, 1955). Concerning the role of the pancreas PNA on the synthesis of digestive enzymes the results published so far are conflicting (Sesso et al, 1955) and although the present results and those observed after hypophysectomy seem to favour the idea that PNA and amylase synthesis are closely related, unpublished data of this laboratory do not confirm this hypothesis.

The cytoplasmatic atrophy in the acinar cells estimated by the increase in DNA concentration, has been observed to be greater in the adrenalectomized male. However, since PNA and amylase concentration were not significantly lowered in the adrenalectomized male in relation to the adrenalectomized female, as one should expect, this point needs further investigation.

In our experiments, significant actions on the absolute and relative pancreas weight have been observed. The relative pancreas weight (mg/100 g of body wt.) of the adrenalectomized cortisone-injected rats was significantly higher than that of the normal rats. It is a known fact that cortisone has an impairing action upon somatic growth (Wells & Kendall, 1940) and that adrenalectomy exhibits a similar action on the body weight (Hartman & Thorn, 1930). The impaired body growth of the adrenalectomized cortisone-injected animals (see Table II) should be accounted for by the action of adrenalectomy and that of cortisone administration. However in spite of its action in impairing the gain in body weight, cortisone administration prevents the involution of the pancreas in adrenalectomized rats and maintains the absolute pancreas weight at the control levels. It appears that the action of cortisone on the pancreas weight is contrary to its action on the body weight.

The total DNA of the adrenalectomized rat pancreas is significantly less ($P < 0.05$) than that of the controls and that of the adrenalectomized cortisone-injected rats. There is no difference between total pancreas DNA of the controls and that of the adrenalectomized rats injected with cortisone. It has been demonstrated that the total DNA of the rat pancreas increases with age (Enesco, 1956). Apparently this did not occur in the adrenalectomized animals. This fact may be taken as an indication that besides cytoplasmic atrophy, adrenalectomy also causes an impairment in cellular reproduction of the pancreas. This might be but a particular aspect of the impaired somatic growth observed after adrenalectomy (Hartman & Thorn, 1930).

The above results also suggest that cortisone administration in the adrenalectomized rats not only prevents the involution of cytoplasmic elements as PNA and amylase, but also does not significantly interfere with the processes responsible for DNA synthesis. Concerning DNA synthesis, with the doses of cortisone used in this report, it seems that the situation in the pancreas is different from that described in the regenerating liver. In this organ cortisone acts as a powerful inhibitor upon the synthesis of DNA. After partial hepatectomy in mice (Roberts, Florey & Joklick, 1952) and rats (Einhorn, Hirschberg & Gellhorn,1954) it has been observed that cortisone promotes the restoration of the weight of the liver mainly by acting upon cytoplasmic growth while in the control animals there is an increased rate of cell multiplication. These experiments have also demonstrated that the hepatic cells of the cortisone treated animals increase

in weight mainly due to an increased infiltration of lipids. Although a thorough chemical examination of the pancreas from the adrenalectomized rats injected with cortisone was not made, it seems that cortisone did not alter the chemical constitution of the pancreas; on the contrary it seems effective as replacement therapy in preventing the cellular involution caused by adrenalectomy.

Another fact observed incidentally in this experiment is that the pancreas weight per g of body weight is higher in the female than in the male of the same age. This fact seems not to have been reported. From unpublished observations we can anticipate that this sexual difference in the relative pancreas weight may be detected in the rat by the end of the first month of post-natal life.

Summary

Adrenalectomy induces cytoplasmic atrophy in the rat pancreas, which is evidentiated by an increase in DNA concentration as well as by a decrease in the concentration of PNA and of amylase. Cortisone injections prevent the involution of the acinar cells as shown by determinations of DNA, PNA and amylase activity concentrations. Adrenalectomy impairs cellular multiplication in the pancreas; cortisone administration on adrenalectomized rats, maintained total DNA at the control levels.

We whish to thank Prof. L. Lison for his invaluable help in the statistical treatment of the results and also for much useful advice and criticism.

References

Abrams, G. D. & Baker, B. L.: *Gastroenterology*, 1954, *27*, 462.
Baker, B. L. & Abrams, G. D.: *Hypohyseal Growth Hormone; Nature and Actions* edited by R. W. Smith Jr., O. H. Gaebler and C. N. H. Long, New York: Blakiston, 1955, page 107.
Baker, B. L. & Brigman, R. M.: *Amer. J. Anat.* 1954, *94*, 363.
Baker, B. L., Reid, N. L. & Thoms, N.: *Amer. J. Physiol.* 1956, *186*, 57.
Barret, J. L., Nishikawara, M. & Haist, R. E.: *Amer. J. Physiol.* 1955, *182*, 35.
Brown, A. H.: *Arch. Biochem.* 1946, *11*, 269.
Bryans, F. E., Kinash, B., Ashworth, M. A. & Haist, R. E.: *Diabetes*, 1952, *1*, 358.
Einhorn, S. L., Hirschberg, E. & Gellhorn, A.: *J. gen. Physiol.* 1954, *37*, 559.
Enesco, M.: *Anat. Rec.* 1956 *124*, 285 (Abstract 199).
Haist, R. E.: *The Hypophyseal Growth Hormone*, page 437 (see above).
Hartman, F. A. & Thorn, G. W.: *Proc. Soc. exp. Biol.* N. Y., 1930, *28*, 94.
Kinash, B. & Haist, R. E.: *Amer. J. Physiol.* 1954, *178*, 441.
Kinash, B. & Haist, R. E: *Canad. J. Biochem. Physiol.* 1955, *33*, 380.
Koster, S.: *Pflüg. Arch. ges. Physiol.* 1930. *224*, 212.
Nishikawara, M., Barret, J., Maykut, M., Sprague, L. & Haist, R. E.: *Fed. Proc.* 1954, *13*, 105.
Pereira, R. S.: *Bull. Soc. Chim. Biol.* 1939, *21*, 827.
Rabinovitch, M.: Thesis University of São Paulo, 1951.
Roberts, K. B., Florey, H. W. & Joklick, W. K.: *Quart. J. exp. Physiol.* 1952, *37*, 239.
Schmidt, G. & Thannhauser, S. J.: *J. biol. Chem.* 1945, *161*, 83.
Scheneider, W. C.: *J. biol. Chem.* 1945, *161*, 293.
Sesso, A., Tramezzani, J. H., Valeri, V. & Migliorini, R.: *C. R. Acad. Sci.* (Paris), 1955, *241*, 775.
Smith, B. W. & Roe, H. J.: *J. biol. Chem.* 1949, *179*, 53.
Wells, B. B. & Kendall, E. C.: *Proc. Mayo Clin.* 1940, *15*, 324.

THE ANABOLIC ACTION OF CORTISONE UPON THE EXOCRINE PANCREAS OF THE SUCKLING RAT (*)

A. Sesso and L. G. Olivério (**)

(Department of Histology [Professor Lucien Lison]. Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brazil.)

It has been demonstrated that the atrophic pancreas of the hypophysectomized rat (Koster, 1950; Bryans et al, 1952; Sesso et al 1955) increases in weight after the administration of large dosis of cortisone or ACTH (Kinash & Haist, 1954; Baker & Abrams, 1955). In the adrenalectomized rat an atrophy of the pancreas was also observed as well as a decrease in the concentration of pentose-nucleic acid and in the amylase activity. In these animals cortisone administration was able to maintain the normal level of these cytoplasmic components (Sesso & Migliorini, 1959). These results strongly indicate that the adrenal cortex plays an important rôle upon the exocrine pancreas activity.

The above experiments however were performed in adult rats whose pancreases had already overpassed the period of intensive mitotic growth which occurs during the first month of post-natal life (Sesso, unpublished data). It is well known that cortisone, besides impairing the rate of body growth, also exhibits an inhibiting effect upon a wide variety of proliferating tissues. [For references see Roberts, Florey & Joklick (1952), Einhorn, Hirschberg & Gellhorn (1954) and Sissons (1955).] Therefore it seemed interesting to study the effect of cortisone upon the pancreas in very young male and female rats.

Methods

Experiment I. — In this experiment, it was used a complete randomized block design, using the litters as blocks.

Preliminary observations showed that the mean body weights of the animals of differents litters of the same age, are somewhat different and it appeared desirable to adapt a technique allowing the statistical control of this factor.

Six litters of the Wistar strain, each one containing four suckling rats were used. The animals were kept with their mothers throughout the experiments.

(*) This work was aided by grants from the Conselho Nacional de Pesquisas and the Rockefeller Foundation.

Received for publication, July 30th, 1958.

Each litter (block) contained two suckling males an two suckling females. One male and one female were injected with cortisone and the two other littermates remained as controls. A preliminary experiment was made in animals of the same age as those used for this report in order to assay the dosis of cortisone that should be used. Daily injections of 250 μg. of cortisone per 10 g. of body weight was found to be a suitable dosis. The hormone was injected daily for nine days in rats which on the first day of injection were 8 days old. The weights of the treated animals and of the controls were recorded daily. At the beginning of the experiment the weights of the animals within each block were alike; differences in the body weight greater than 4 g. among the animals of the same litter were not observed. On the day following the last injection the animals were killed by ether inhalation. The pancreas was excised, dissected from lymph nodes and adipose tissue and weighed. Small pieces of organs were fixed in Champy fluid and in Susa mixture (without trichloroacetic acid) for three to six hours. Sections from Champy fixed pancreas were stained by the acid fuchsin according to Altmann and sections from the material fixed in formol, sublimate, acetic acid mixture, were stained by Einarson gallocyanin and also by Masson's trichrome stain.

Experiment II. – To study the earlier changes and the subsequent cytological modifications in the pancreas after cortisone administration, suckling rats aged of nine days were injected with the hormone in the dosis already indicated. Autopsy was performed 6, 12 and 24 hr after the first injection. Daily injected animals were sacrificed 2, 3 and 4 days after the first series of injections. In each instance, two cortisone injected rats and two littermates subcutaneously injected with saline were used.

Results

Experiment I. – Rats treated with cortisone for 9 days

The pancreases of the cortisone injected animals presented a remarkable macroscopic difference from those of the controls. While in the latter animals the pancreas was friable and of a white-grey colour, in the former it was more consistently of a yellow-white colour and definitely larger. The quantitative findings are recorded in Table I.

TABLE I

	Control Male	Control Female	Cortisone-injected male (nine days of injections)	Cortisone-injected female (nine days of injections)
Nº of animals	6	6	6	6
Mean Initial body weight (g) ...	13.7	12.2	13.8	13.3
Mean Final body weight (g)	24.1	22.9	18.8	18.1
Mean Pancreas weight (mg)	53,1	54.2	86.7	96.1
Mean Pancreas weight mg/100 of body weight	225	238	462	533

The results were submitted to the analysis of variance. In table II the analysis of variance of the final body weight of the experimental animals is given.

TABLE II

Analysis of variance performed on the data of final body weight

Source of variation	Sum of squares	D. F.	Mean Square	F.
Action of cortisone	149.00	1	149.00	57.7 (*)
Difference due to sex	5.23	1	5.23	2.0
Interaction: Sex x cortisone	0.33	1	0.33	0.1
Between blocks	172.82	5	34.56	13.4 (*)
Residual Error	38.74	15	2.58	
TOTAL	366.12	23		

(*) Significant at the probability level of 1 %.

It is concluded from the above analysis that cortisone causes highly significant reduction in the gain of body weight ($F = 57.7$). The analysis also shows a highly significant difference between the litters (blocks) indicating that the growing rate of different litters varies.

Since the analysis shows that no significant differences are to be found between male and female, it is allowed to consider the treatment regardless of sex.

The difference between the mean final body weight of the cortisone treated and of the control animal is $\frac{1}{2}(24.1 + 22.9) - (18.8 + 18.1) = 5.05$ g.

From the mean squares for the error, the limits of the confidence interval for this difference may be calculated:

$$5.05 - t\sqrt{\frac{2}{12} \times 2.58} \qquad 5.05 + t\sqrt{\frac{2}{12} \times 2.58}$$

The t value for 15 degrees of freedom at the 0.05 critical values is $t = 2.13$ and therefore the limits of the 95 % confidence interval for the differences are 6.45 and 3.65 g. Converting these values into percentages it may be seen that cortisone treatment caused a mean decrease of 21.5 % in the body weight with a 95 % confidence; that the decrease is not less than 15.5 % and not more than 27.4 %.

In table III the analysis of variance made on the results of pancreas weight is given.

TABLE III

Analysis of variance performed on the data of pancreas weight

Source of variation	Sum of squares	D. F.	Mean Square	F.
Action of cortisone	855.793	1	855.793	81.3 (*)
Difference due to sex	16.748	1	16.748	1.6
Interaction: Sex x cortisone	10.417	1	10.417	1.0
Between blocks	65.652	5	13.130	1.2
Residual Error	157.891	15	10.527	1.2
TOTAL	1.106.501	23		

(*) Significant at the probability level of 1 %.

As it may be drawn from the analysis of variance, cortisone causes a highly significant increase in the weight of the pancreas. Neither sexual differences in the weight of the pancreas nor a different action of cortisone (interactions: sex x cortisone) was observed.

The mean pancreas weight of the controls and of the treated rats were 53.6 and 91.4 mg. respectively. The confidence interval for the difference of the these means has been calculated as indicated above, with the following conclusion: there is a 95 % confidence that cortisone increased the mean pancreas weight of a value between 53.4 % and 87.4 % of that of the controls, with a mean increase of 70,4 %.

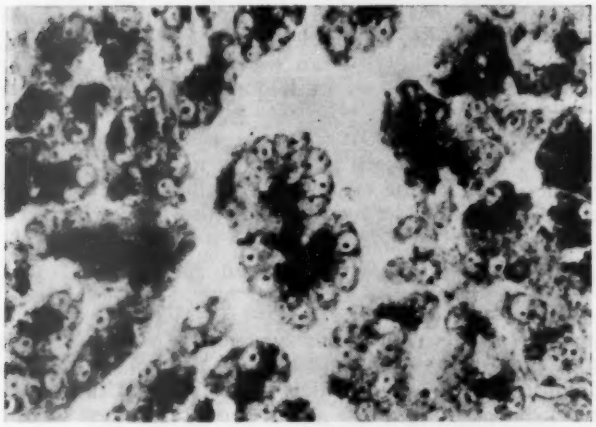
TABLE IV

Analysis of variance performed on the data of the relative pancreas weight

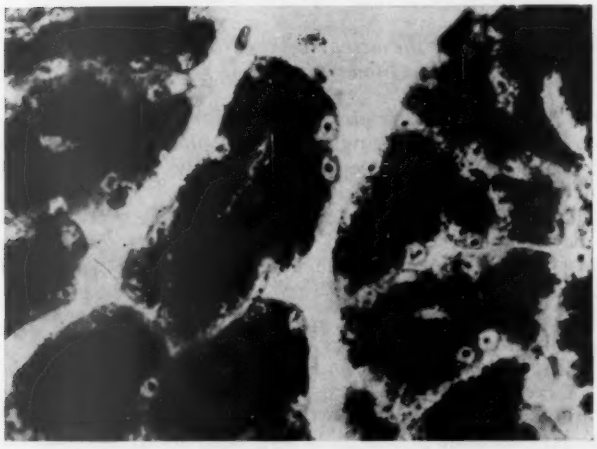
Source of variation	Sum of squares	D. F.	Mean Square	F.
Action of cortisone	416.594	1	416.594	170.2 (*)
Difference due to sex	9.362	1	9.362	3.8
Interaction: Sex x cortisone	4.004	1	4.004	1.6
Between blocks	24.685	5	4.939	2.0
Residual Error	36.718	15	2.448	
TOTAL	491.373	23		

(*) Significant at the probability level of 1 %.

The mean increase in the relative pancreas weight was of 114.2 % in relation to the controls, the lower and upper limits of the confidence interval of this increase at the 5 % probability level being 95.6 and 132.9 % respectively.



1 A.



1 B.

FIG. 1 A. — *Pancreas of a 18 —day— old rat, given the suspending fluid alone for 9 days, showing the acini and zymogen granules as they are frequently seen at this age. Champy fixation. Altmann's acid fuchsin x 643.*

FIG. 1 B. — *This figure shows the striking enlargement of the acini, filled with zymogen granules, seen in a 18 day-old rat given cortisone for 9 days. Note the enlargement of nucleoli. Champy fixation. Altmann's acid fuchsin x 643.*

Cytological modifications in the acinar cells of rats injected with cortisone for nine days

The cytological picture of the exocrine pancreas of a normal rat at the 18th day of age is briefly the following: the acini and the acinar cells are smaller

than those of the adult rat. Zymogen granules occupies the middle and the apical zone of the cells and they are fewer in number than in the adult pancreas.

The acinar cells of the cortisone injected rats were greatly enlarged. The nucleoli are smaller than in the adult. Mitoses are frequently seen in the acinar cells at this age.

The acinar cells of the cortisone injected rats were greatly enlarged. The cytoplasm was completely filled with zymogen granules. In most acini the basophilic basal zone was hardly seen, for the cytoplasm contained practically but secretory granules (Fig. 1 A and 1 B). The nucleoli were also greatly enlarged.

Experiment II. — Rats treated with cortisone for 6, 12 and 24hr and for 2, 3 and 4 days

No clear cut differences were observed grossly between the pancreas of the animals previously injected with cortisone for 6 and 12hr and those of the controls. 24hr after cortisone administration it was seen that the colour and the consistency of the pancreas have changed toward the aspect already described for the animals injected during nine days; the macroscopic changes became conspicuous in the animals injected with cortisone for 2, 3 and 4 days.

Cytological modifications in the acinar cells 6, 12 and 24hr after cortisone

There was no definite cytological response 6hr after cortisone administration. However as soon as 12hr after a single injection of the hormone the following was observed: moderately enlarged cytoplasm, slightly more prominent nucleoli and slightly increased basophilic basal substance (BS).

The cytoplasmic enlargement was more pronounced in the pancreas of rats killed 24hr after the hormone administration. However the most striking cytological modification was the increase of the basophilic substance (BS) of the acinar cells (Fig. 2 A and 2 B).

Cytological modifications in the acinar cells, of rats injected with cortisone for 2, 3 and 4 days

The pancreas of animals injected with cortisone for two days exhibited marked cytological changes: nucleoli greatly enlarged, with its dimensions far beyond those observable on animals injected with cortisone for shorter periods; after 3 and 4 days of treatment these changes were still more intense. However it is during the first 48 hours that the cytological modifications due to cortisone injection are most striking. After this period the cytological modifications, although present, are not so dramatic.

In animals injected for 2 days, the basal basophilia seemed to be more intense than that of the controls, but it was not so strong as that observed 24hr after cortisone.

After 4 days of cortisone administration the secretory granules completely filled the cytoplasm and the BS was restricted to a thin crescentic zone under the nucleus (Fig. 2 C). It appears that after cortisone administration there is an initial (12-24hr) period in which PNA, as revealed by basophilia staining, seems to increase, with a concomitant increase in the number of secretory

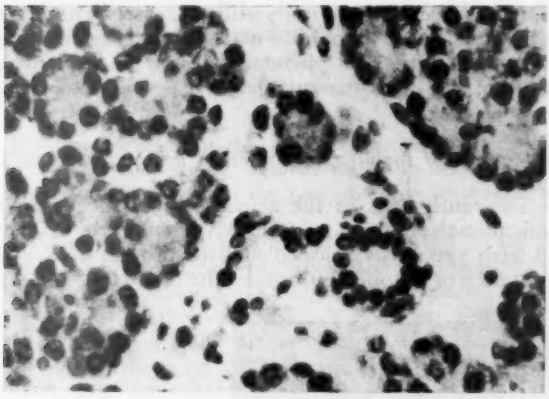


FIG. 2 A. — *Pancreas of a 10-day old control rat, showing the small amount of basophilic material at the basal zone of the acinar cells. Gallocyanin x 643.*

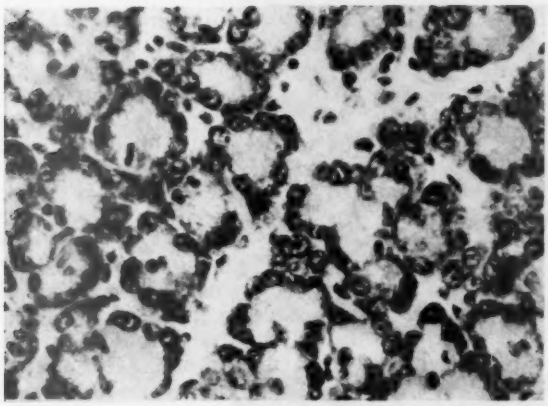


FIG. 2 B. — *Pancreas of a 10-day old rat, which has received a single injection of cortisone, 24 hr prior to the autopsy. Note the greater basophilia of the acinar cells in relation to the control. Two mitosis are also seen. Gallocyanin x 643.*

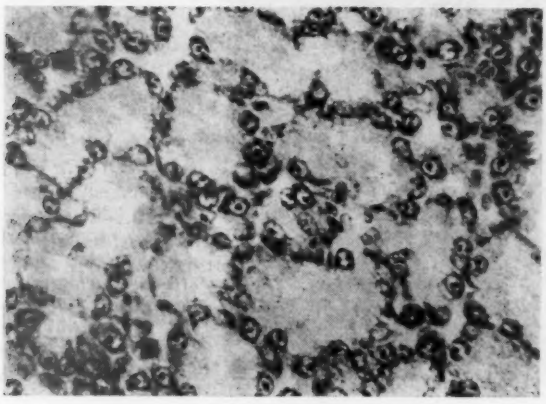


FIG. 2 C. — *Pancreas of a 13-day old rat given cortisone during 4 days. Note the enlargement of the acini and the decreased basophilic zone in relation to the cells of fig. 2 B. Gallocyanin x 643.*

granules. Later on, the amount of secretory granules still increases, without a parallel increase in the visible amount of basophilic material; on the contrary a diminution of the PNA seems to occur. This phenomenon may be explained, at least in part, by the dilution of the BS in the enlarged acinar cells cytoplasm.

Mitoses in pancreas acinar cells of suckling rats injected with cortisone

Although mitoses were frequently seen in the acinar cells of the controls rats, a normal occurrence for animals at this age (Fig. 3 A), it became evident that the animals which had been previously injected with cortisone for 12 and 24hr exhibited a far greater number of mitotic figures than the controls (Fig. 3 B).

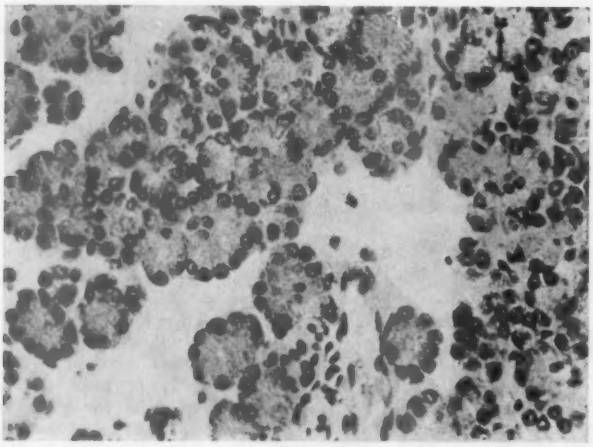


FIG. 3 A. — *Same specimen as fig. 2 A. Shows mitosis, a normal occurrence at this age. Gallocyanin x 384.*

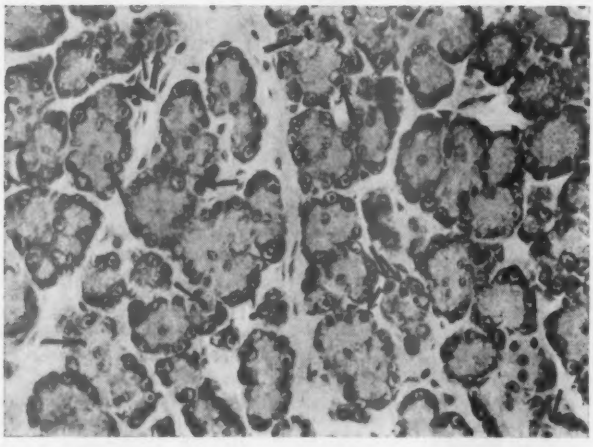


FIG. 3 B. — *Same specimen as fig. 2 B. Demonstrates the great number of mitosis. The increase in the basophilic material, as compared to fig. 3 A, may also be seen. Gallocyanin x 384.*

In the animals treated for 6hr, 2, 3 and 4 days, no differences in relation to the controls could be ascertained by simple inspection.

Discussion

The increase in the pancreas weight, concomitantly with a decreased rate in the gain of body weight, indicates that the action of cortisone on the pancreas is contrary to the one observed in several organs and in the organism as a whole.

However it may be doubted if the effect on the pancreas is actually anabolic, since the accumulation of zymogen granules could be the result of an impairing action of cortisone on the extrusion mechanism of acinar cells without affecting the rate of synthesis of secretory granules. This seems not to be the case, since cortisone has already proved to be effective in influencing PNA and digestive enzyme synthesis on the pancreas in adrenalectomized rat (Sesso & Migliorini, 1959). On the other hand, present cytological evidence suggests that active synthesis of cytoplasmic material does occur. Positive signs of an increase in the rate of synthesis of secretory products are the increased nucleolar volume and the basal basophilia modifications which previous cytochemical observations on pancreas and other glands showed to be morphological manifestation of increased metabolic activity. References on the subject can be found in Oram's monograph (1955).

The modifications observed after cortisone administration are in some way comparable to those seen after thyroxine injections. Thyroxine administration to the suckling rat causes a remarkable increase in pancreas weight, and, after a few days, the cytological aspect of the exocrine pancreas is similar to that of an adult rat. The acinar cells exhibited signs of an intense synthesis of cytoplasmic material: enlargement of the nucleoli, greater basal basophilia, accumulation of zymogen granules (Sesso, 1957). In order to evaluate the significance of these findings it is worthwhile to remember that during the first month of post-natal life, the acinar cells of the rat pancreas undergo marked modifications (Sesso, 1957). The initially small amount of BS present in the cells during the first post-natal week, undergoes an increase from the 15th day on. Concomitantly, the nucleoli enlarge and the amount of zymogen granules increases. This will be referred to as the cytological "maturation" of the cell. The "mature" cytological picture characteristic of the adult is only attained by the 30th day of post-natal life. At this time the acini are broader, the nucleoli are larger and the cytoplasm contains more BS and more zymogen granules than in earlier ages.

All the above events are brought about earlier by the action of cortisone and also by that of thyroxine. Since these two hormones play an important role upon the normal activity of the adult rat pancreas (Nishikawara, et al., 1954; Haist, 1955; Sesso & Valeri, 1958; Sesso & Migliorini, 1959), the results obtained in suckling rats suggest that during the development of the pancreas, cortisone and thyroxine secretion plays an important role in the cytoplasmic differentiation of the acinar cells. The assumption that thyroxine and cortisone are related to pancreas acinar cells "maturation" is supported, at least concerning the thyroid hormone, by the following facts: about the 15th day of post-natal life the cytologic picture of the pancreas begins to change markedly, as referred above. At this time, more thyroid hormone seems to be released into the blood stream

(Phillips & Gordon, 1955 a); at 30-40 days, the levels of plasma thyroid hormone seems to increase still further (Phillips & Gordon, 1955 b). These hormonal changes parallel quite closely the cytological modifications described in the pancreas during the same period. Unfortunately there is no data regarding the blood levels of adrenal cortical hormones during early post-natal life in the rat. Although the available data suggest that in earlier post-natal periods the adrenal cortical hormones secretion is not yet fully established (Landman & Deane, 1955; Jailer, 1950; Jailer, 1951; Eiseinstein & Hartroff, 1957), determination of these hormones in the blood of growing rats are necessary to allow conclusions concerning the onset of their peripheral action in early post-natal life. The fact that cortisone administration in intact rats weighing about 131 g did not cause any appreciable alteration in the absolute pancreas weight (Kinash & Haist, 1954), could be taken as an indication that after the "maturation" period this organ is no more sensitive to exogenous cortisone. This is an indirect confirmation that cortisone like hormones act on "maturation" of the cell.

Thyroxine seems to act on the weight of the pancreas of the suckling rat, not only by enlarging the existing cells, but also by stimulating cellular multiplication, as indicated by an increase in total desoxyribonucleic acid after thyroxine administration (Mandel, Jacob & Mandel, 1954).

It is quite possible that cortisone acts in the same way. Although cortisone has an inhibitory effect on the proliferation of several tissues, this action seems not to be general. The mitoses in the intestinal epithelium of the mouse are not affected by cortisone (Roberts, Florey & Joklick, 1952). Our present results indicate the possibility of an stimulating action upon mitosis. The increased number of mitotic figures seen 12 and 24hr after a single injection of the hormone suggests that mitosis has been induced in the suckling rat pancreas by cortisone.

Cortisone has already proved to be effective in causing premature histological differentiation in other organs, as the duodenum (Moog, 1953; Moog & Thomas, 1955), kidney (Ross & Goldsmith, 1954) and lower incisors (Goldsmith & Ross, 1956; Domm & Leroy, 1956). As in the case of the pancreas, it remains to be verified whether this action of cortisone is direct or is mediated through other endocrine glands.

The actions of cortisone and of thyroxine upon the pancreas of the suckling rat does not seem to be identical. In our experience, the acinar cell enlargement due to increased zymogen granules storage is more pronounced after cortisone than after thyroxine administration. However it must be kept in mind that degrees in the response of the targed tissue, the exocrine pancreas, in this case, depends mostly on the dosage, extension of the treatment, age of the animals, and on general conditions of the suckling rats, which varies from litter to litter. In order to compare the effects of thyroxine and cortisone, experiments are being carried out on suckling rats, of the same litter, using different dosis of the hormones.

Summary

Eight days-old suckling rats, were injected with cortisone during nine days. Cortisone caused a reduction in the gain of body weight and increased the mean pancreas weight which was 70.4 % higher than controls. Marked cytological changes were observed: pronounced cytoplasmic enlargement with ac-

cumulation of zymogen granules; increase of the nucleolar volume; reduction of the basophilic basal zone. As soon as 12-24hr after a single injection of cortisone, nucleolar modifications and a slight cytoplasmic enlargement could be observed. During this period the basophilic substance of the acinar cells increased in amount; later on, the visible amount of this substance decreased. After a single injection of cortisone into 10 day old rats, an increased number of mitotic figures were observed in the pancreas acinar cells.

We are indebted to Prof. L. Lison for his valuable help in the statistical treatment of the results.

References

BAKER, B. L. & ABRAMS, S. G.: *Hypophyseal Growth Hormone; Nature and Actions* edited by R. W. Smith Jr., O. H. Gaebler and G. N. H. Long, N. Y. Blakiston, 1955, page 107.

BRYANS, F. E., KINASH, B., ASHWORTH, M. A. & HAIST, R. E.: *Diabetes*, 1952, *1*, 358.

DOMM, L. & LEROY, P.: *Anat. Rec. 124*, 281 Abstr. 28, 1956.

EINHORN, S. L., HIRSCHBERG, E. & GELLHORN, A.: *J. gen. Physiol.* 1954, *37*, 559.

EISEINSTEIN, A. B. & HARTROFT, P. M.: *Endocrinology*, 1957, *60*, 634.

GOLDSMITH, E. D. & ROSS, L.: *Acta Endocrinol.* 1956, *22*, 23.

HAIST, R. E.: *Hypophyseal Growth Hormone; Nature and Actions* edited by R. W. Smith Jr., O. H. Gaebler and G. N. H. Long, N. Y. Blakiston, 1955, page 437.

JAILER, J. W.: *Endocrinology*, 1950, *46*, 420.

JAILER, J. W.: *Endocrinology*, 1951, *49*, 826.

KINASH, B. & HAIST, R. E.: *Amer. J. Physiol.* 1954, *178*, 441.

KOSTER, S.: *Pflug. Arch. ges. Physiol.* 1950, *224*, 212.

LANDMAN, A. J. & DEANE, H. W.: *Anat. Rec. 122*, 481 (Abst.), ...-1955.

MANDEL, L., JACOB, M. & MANDEL, P.: *J. Physiologie*, 1954, *46*, 456.

MOOG, F.: *J. exp. Zool.* 1953, *124*, 329.

MOOG, F. & THOMAS, E. R.: *Endocrinology*, 1955, *36*, 187.

NISHIKAWARA, M., BARRET, J., MAYKUT, M., SPRAGUE, L. & HAIT, R. E.: *Fed. Proc.* 1954, *13*, 105.

ORAM, V.: *Acta Anatom. Supplementum*, *25*, 1955, 23 = 1 AD Vol.

PHILLIPS, J. B. & GORDON, A. S.: *Anat. Rec.* 1955 a, *123*, 487.

PHILLIPS, J. B. & GORDON, A. S.: *Anat. Rec.* 1955 b, *122*, 477 (Abst.).

ROBERTS, K. B., FLOREY, H. W. & JOKLICK, W. K.: *Quart. J. exp. Physiol.* 1952, *37*, 239.

ROSS, L. & GOLDSMITH, E. D.: *Amer. J. Physiol.* 1954, *179*, 667.

SESSO, A., TRAMEZZANI, J. H., VALERI, V. & MIGLIORINI, R.: *C. R. Acad. Sci.* (Paris), 1955, *241*, 775.

SESSO, A.: *C. R. Acad. Sci.* (Paris), 1957, *245*, 1832.

SESSO, A. & VALERI, V.: *Exptl. Cell Res.* 1958, *14*, 201.

SESSO, A. & MIGLIORINI, R.: *Acta physiol. lat.-amer.*, 1959 (in press).

SISSONS, H. A.: *J. Anat.* 1955, *89*, 69.

ENDOCRINE CHANGES IN HEMIDECORTICATE RATS

MIGUEL R. COVIAN, RENATO H. MIGLIORINI and JUAN H. TRAMEZZANI

(Department of Physiology, Ribeirão Preto School of Medicine, University of São Paulo, Brazil.)

IT HAS been observed that hemidecorticate rats show some particular features: transient arterial hypertension (Covian and Houssay, 1955), hyperphagia (Covian, Hartmann and Granel, 1942) delayed urine excretion (Covian and Uranga, 1953) and changes in carbohydrate metabolism (Covian, Migliorini and Berezin, 1958).

There is increasing evidence on the rôle played by the hypothalamus in the regulation of the endocrine system through the hypophysis, but little is known on the participation of other parts of the central nervous system in this regulation.

In an attempt to study this problem, the present paper deals with the changes observed in the weight of endocrine glands after hemidecortication in white rats.

MATERIAL AND METHODS

First group: Adult white male and female rats weighing 150-200 g were used. Hemidecortication was performed under nembutal anesthesia (4 mg per 100 g intraperitoneal) in the following way: after a midline incision and opening of the skull, the cortical tissue of one side was sucked away with a small glass cannula using light suction. Figure 1 shows the dorsal and ventral views of one brain in which the operation has been performed: all cerebral tissue to the thalamus and coliculli was removed. Control rats underwent a sham operation consisting in all steps of the surgical procedure except removal of cortical tissue.

Second group: Adult white male and female rats (150-200 g) were hemidecorticated and castrated in a one stage operation. Controls of both sexes were castrated and underwent a sham brain operation.

Three to four months after the operation an autopsy was performed in the rats which were in good health; 13 males and 12 females of the first group and 8 females and 8 males of the second one, with their correspondent controls were thus studied. The organs were weighed on a precision balance, 0.1 mg step, and the results expressed in mg per cent of body weigth. The statistical analysis considered the mean value, its standard error and the fiducial limits of the mean for $p = 5\,\%$. In every group the results were submitted to an analysis of covariance.

Received for publication, July 30th, 1958.

The thymus was considered in a special group formed by 16 males and 10 females after one year of hemidecortication, and their correspondent controls of the same age.

The data obtained regarding adrenals and ovaries of 6 female mice after one year of hemidecortication are added.

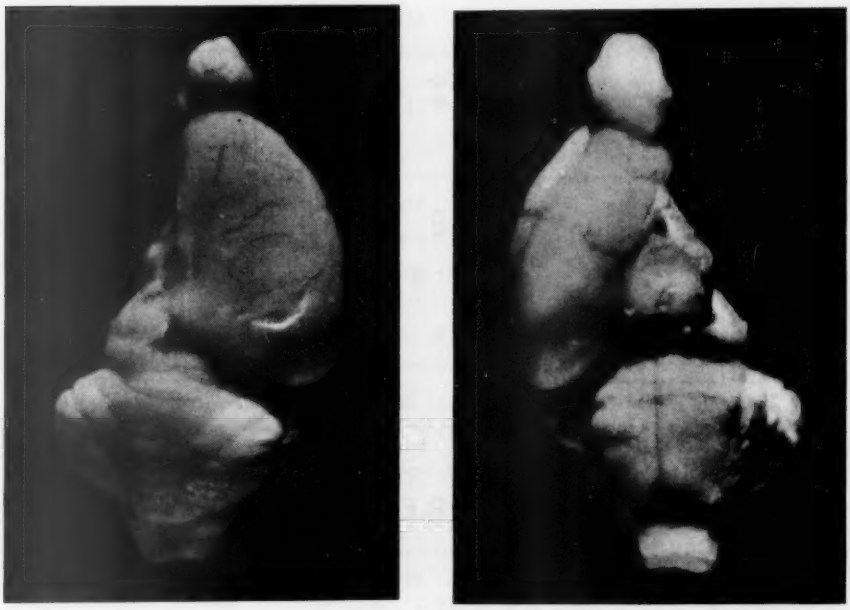


FIG. 1.—*Photographs to illustrate the hemidecortication performed. On left: dorsal view. On right: ventral view.*

RESULTS

Adrenals: The results obtained in hemidecorticate rats and their controls are shown in Table I and Figure 2. The adrenal glands of 12 male hemidecorticate rats are lighter than their 12 controls; the mean value being 10.32 mg % $\pm$ 0.43 for the hemidecorticate rats and 15.06 mg % $\pm$ 0.76 for the control rats. The difference is highly significant. In females, the results show that the hemidecorticate ones have heavier adrenals than their 11 controls, the values being 26.84 mg % $\pm$ 1.02 and 19.36 mg % $\pm$ 0.77 respectively. The difference is also highly significant.

This difference in the weight of adrenal glands was not present in the rats of the second group as can be observed in Table II and figure 3. In hemide-

corticate-castrate male rats the values are: 19.4 mg % ± 1.04 while in 7 castrate males they are: 20.0 mg % ± 1.04. There is no significative difference. The same is true in female rats, where values are: 24,3 mg % ± 1.3 for hemidecorticate-castrate rats and 24 mg % ± 1.2 for control castrate rats.

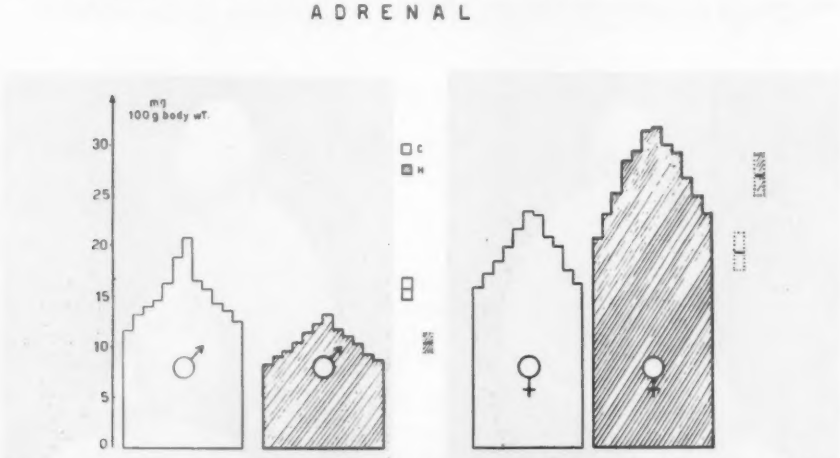


FIG. 2. — *Adrenal weight (mg/100 g body wt.) in male and female hemidecorticate (shaded areas) and control rats (white areas). The column on the right side of every group indicates mean and the fiducial limits of the mean. The difference in both groups is significant. C, control; H, hemidecorticate.*

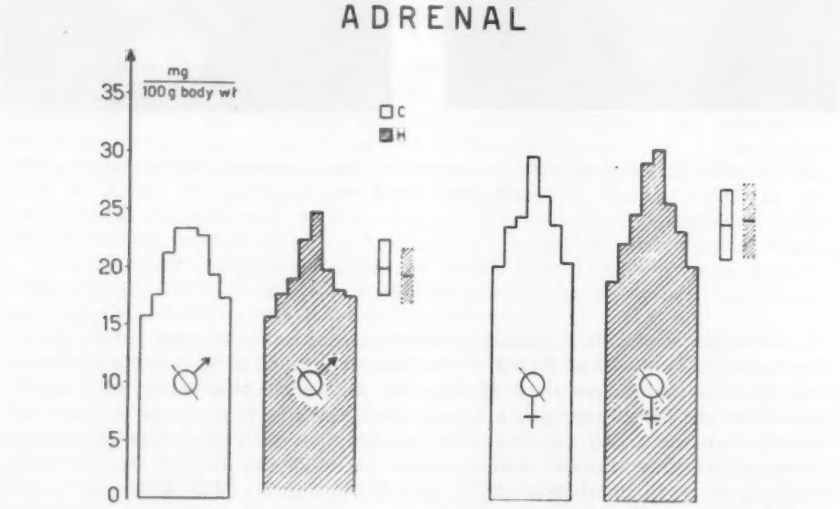


FIG. 3. — *Adrenal weight (mg/100 g body wt.) in castrate hemidecorticate rats (shaded areas) and castrate controls (white areas). See legend of Fig. 2. There is no significative difference between both groups.*

TABLE I

Mean values (mg/100 g body wt) of endocrine glands in hemidecorticate rats — three to four months after the operation — and control rats of both sexes

Organ	Sex	Control	Hemidecorticate	
Adrenal	Male	n = 12 15.01 ± 0.76 (*)	n = 12 10.32 ± 0.43	$P < 0.01$
	Female	n = 11 19.36 ± 0.77	n = 12 26.84 ± 1.02	$P < 0.01$
Thyroid	Male	n = 12 10.35 ± 0.9	n = 13 6.41 ± 0.31	$P < 0.01$
	Female	n = 11 10.35 ± 0.76	n = 12 6.48 ± 0.35	$P < 0.01$
Hypophysis	Male	n = 12 2.5 ± 0.09	n = 13 2.4 ± 0.09	$P > 0.05$
	Female	n = 10 4.06 ± 0.23	n = 12 4.97 ± 0.27	$P < 0.1$
Thymus (**)	Male	n = 16 44.43 ± 5.24	n = 16 11.45 ± 2.68	$P < 0.01$
	Female	n = 10 75.66 ± 15.82	n = 10 35.54 ± 8.75	$P < 0.05$

(*) mean ± standard error of mean.
(**) one year after operation.
n = number of rats.

Thyroid: The changes in weight of the thyroid gland in rats of the first group are shown in Table I and Figure 4. In male as well as in female hemidecorticate rats the thyroid weights less than the controls; the difference being highly significant. The values obtained in 13 male hemidecorticate are: 6.41 mg % ± 0.31 and in 12 controls, 10.35 mg % ± 0.9. In 12 hemidecorticate female rats a mean value of 6.48 mg % ± 0.35 was found and 10.35 mg % ±0.76 in their 11 controls.

This difference disappeared with castration as can be seen in Table II and Figure 5, where 8 hemidecorticate-castrate males have a mean value of 8.9 mg %

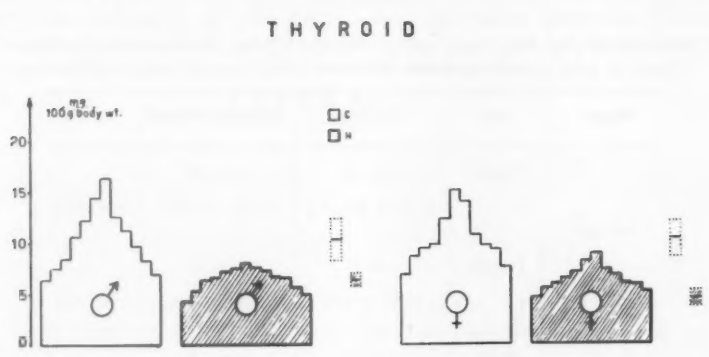


FIG. 4. — *Thyroid weight (mg/100 g body wt.) in hemidecorticate (shaded areas) and control rats (white areas). See legend of Fig. 2. The difference is significant.*

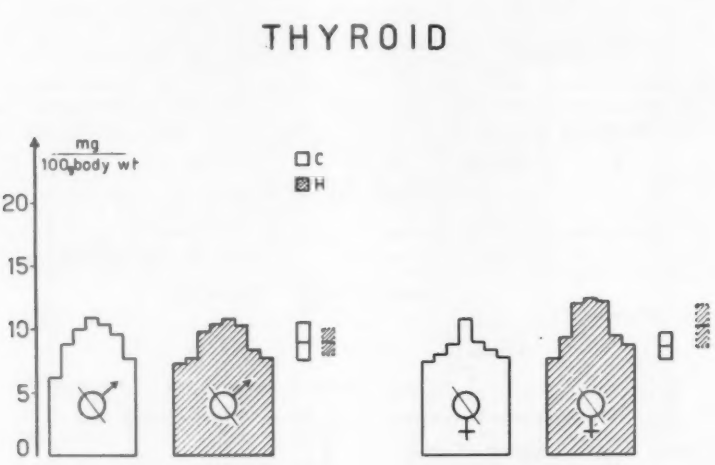


FIG. 5. — *Thyroid weight (mg/100 g body wt.) in hemidecorticate castrate rats (shaded areas) and castrate controls (white areas). See legend of Fig. 2. The difference is not significant.*

± 0.05 and their 7 controls have a mean of 9.0 mg % ± 0.61. The analysis of covariance does not show any significative difference. In female rats, 7 hemidecorticate-castrates shown a mean value of 10.2 mg % ± 0.71 and for their 7 controls the values are: 8.6 mg % ± 0.41.

Hypophysis: The hypophysis of 13 hemidecorticate male rats —Table I,

TABLE II

Mean values (mg/100 g body wt) of endocrine glands in hemidecorticate — castrate rats — three to four months after the operation — and castrate rats of both sexes

Organ	Sex	Castrate	Hemidecorticate Castrate	
Adrenal	Male	n = 8 20.0 ± 1.04 (*)	n = 8 19.4 ± 1.04	P > 0.05
	Female	n = 7 24.0 ± 1.2	n = 8 24.3 ± 1.3	P > 0.05
Thyroid	Male	n = 7 9.0 ± 0.61	n = 8 8.9 ± 0.50	P > 0.05
	Female	n = 7 8.6 ± 0.41	n = 7 10.2 ± 0.71	P > 0.05
Hypophysis	Male	n = 8 5.4 ± 0.22	n = 8 5.1 ± 0.10	P > 0.05
	Female	n = 8 5.4 ± 0.20	n = 7 5.6 ± 0.49	P > 0.05
Thymus	Male	n = 8 169 ± 12.3	n = 7 98 ± 8.6	P < 0.01
	Female	n = 8 152 ± 10.8	n = 8 110 ± 10.9	P < 0.05

n = number of rats.
(*) mean ± standard error of mean.

Figure 6— do not show any significative difference ($p > 0.05$) when compared with the hypophysis of 12 controls as is revealed by the values obtained: 2.4 mg % ± 0.09 and 2.5 mg % ± 0.09 respectively. On the contrary, 12 female operated rats show a hypophysis a little heavier than 10 controls, the values being, 4.97 mg % ± 0.27 for hemidecorticates and 4.06 mg % ± 0.23 for controls. However the statistical analysis gave $0.05 < p < 0.1$.

In the second group —Table II, Figure 7— there is no difference between males, but that observed in female rats has disappeared as shown by the fol-

lowing values: 5.6 mg % ± 0.49 for hemidecorticate-castrate and 5.4 mg % ± 0.20 for castrated control rats.

Thymus: The results obtained in 16 hemidecorticate males and 10 hemidecorticate females one year after operation and their controls are represented in Table I and Figure 8. There is a great difference between males ($p < 0.001$), the

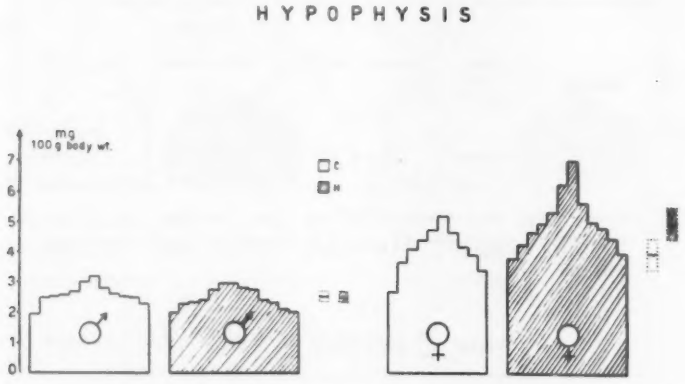


FIG. 6.—*Hypophysis weight (mg/100 g body wt.) in hemidecorticate (shaded areas) and control rats(white areas). See legend of Fig. 2. The difference is only significant for the female group.*

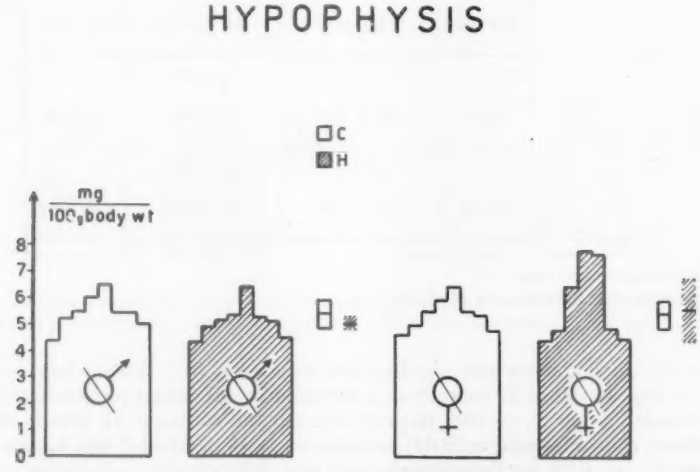


FIG. 7.—*Hypophysis weight (mg/100 g body wt.) in hemidecorticate castrate rats (shaded areas) and castrate controls (white areas). See legend of Fig. 2. No significative difference is observed in either group.*

mean value being 11.45 mg % ± 2.68 for hemidecorticates and 44.43 mg % ± 5.24 for controls. Regarding female rats, the difference is also significant ($p < 0.05$), but not so great as in males. The values — obtained were:
35.54 mg % ± 8.75 for hemidecorticates and 75.66 mg % ± 15.82 for controls.

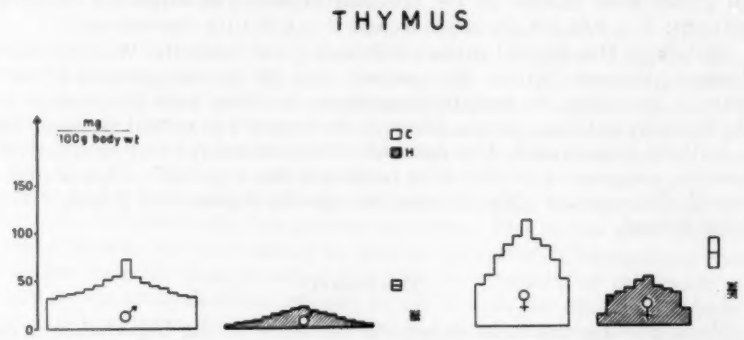


FIG. 8. — *Thymus weight (mg/100 g body wt.) in hemidecorticate (shaded areas) and control rats (white areas). See legend of Fig. 2. The difference is significant in males and females.*

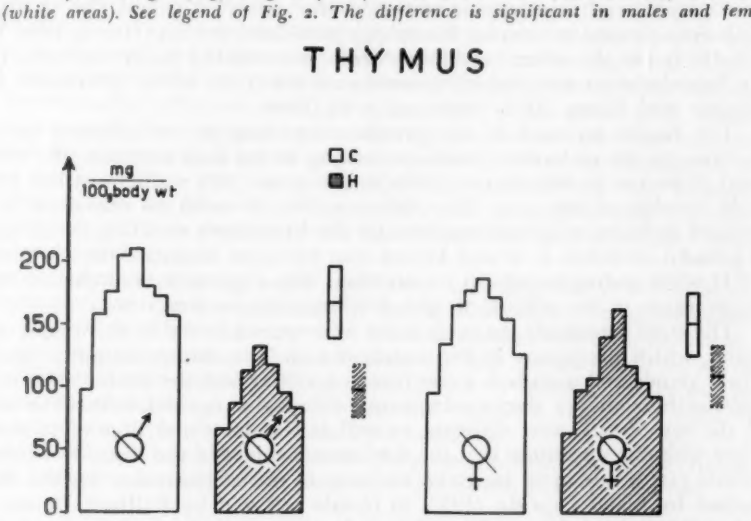


FIG. 9. — *Thymus weight (mg/100 g body wt.) in hemidecorticate castrate rats (shaded areas) and castrate controls (white areas). See legend of Fig. 2. The difference is significant in both groups.*

In the second group —Table II, Figure 9— the difference in both sexes does not disappear after castration, but, as in the previous group, this difference was greater in males. The mean value of 7 hemidecorticate castrate males is 98 mg % ± 8.6 and for 8 castrate males is 169 mg % ± 12.3. The difference is highly significant. In 8 hemidecorticate castrate females the mean value is 110 mg % ± 10.9 and for their 8 castrate controls is 152 mg % ± 10.8. The difference is also significant.

Sexual organs: No significative difference was found in any group regarding testes, seminal vesicles, prostate, ovaries and uterus.

Mice: The adrenals and ovaries of 6 female mice after one year of hemidecortication were compared with 6 female controls of the same strain and age. Both glands were heavier in the operated animals the difference being highly significant: $P > 0.01$ for the adrenals and $P > 0.001$ for the ovaries.

Histology: Histological studies, still under way, with the Weigert and silver techniques, showed that on the operated side all the components of the stria terminalis, including the amigdale-hypothalamic fibers, have disappeared as well as the fimbrial and hippocampic fibers of the fornix. The medial forebrain bundle was partially degenerated. The external cortico-habenular and external-olfatory-habenular components of the stria terminalis have partially disappeared. The mammillothalamic tract (Vicq-D'Azyr) was greatly degenerated (Insua, Florit and Velasco Suarez).

Discussion

Many investigators have shown the influence of the hypothalamus on the adenohypophysis. Studies have been made on the secretion of adrenocorticotrophic, gonadotrophic and thyrotrophic hormones after electrical stimulation or electrolytic lesions. Several reviews on this subject have been written (Harris, 1948, 1950, 1952, 1955). On the other hand the observations relating brain structures other than hypothalamus and endocrine system are relatively scarce (Hemphill 1944, Schreiner and Kling, 1954, Anderson et al. 1948).

The results reported in the present paper suggest an influence of these structures on the endocrine glands. According to the data obtained, the normal sexual difference in the adrenal glands'weight is not only maintained but increased in hemidecorticate rats. The difference found could be explained by an increased secretion of gonadotrophins by the hypophysis resulting in an increased gonadal secretion. It is well known that estrogens stimulate the secretion of ACTH while androgens inhibit its secretion. This suggestion is supported by the disappearance of the adrenal weight difference in castrated rats.

The same hypothesis can explain the difference observed in the weight of the thyroid, which disappears in the castrated rats. The interrelationship between thyroid glands and gonads is a controversial matter and the results obtained by several authors are not always coincident, perhaps due to the fact that the strains and the age of rats, were different as well as the doses and time of treatment. In line with our suggestion that the diminished weight of the thyroid of hemidecorticate rats is due to an increased secretion of sexual hormones, are the results obtained by Bialet Laprida (1933) in female rats and by Bulliard, Delsuc and Moday (1941) in male rats. The first author found that daily injections of folliculine during two or more months to adult female rats (150-175 g body weight) resulted in a fall of the weight of thyroid glands. This fact was confirmed by Franck (1937) in guinea pigs. Benoit and Aren (1931) found that the thyrotrophic action of hypophyseal extracts is diminished by folliculine and the same fact has been observed by Starr (1933) regarding the thyrotrophic activity of the adenohypophysis.

On the other hand, Bulliard, Delsuc and Moday by injecting testosterone to adult male rats (220-235 g body weight) during 116 days obtained also a marked reduction of thyroid's weight.

The increased weight of the hypophysis of female hemidecorticate rats as compared to their controls and the disappearance of this difference in castrate rats support our suggestion of an increased secretion of estrogens in hemidecorticate female rats. Hohlweg (1934) found a macroscopic enlargement of the anterior lobe following the injection of folliculine.

The thymus showed a striking involution in rats of both sexes after one year's operation the difference being greater for males. Chiodi (1938) has shown that sexual hormones determined a marked atrophy of the thymus in rats and this fact is in accordance with our assumption. However, as the thymus is an end-organ in stress reactions, a temporary or permanent hyperadrenocortical function has to be taken into account, considering that hemidecorticate rats are more active and excitable than the normal ones. In rats of the second group, the thymus remained smaller in hemidecorticate castrates than in their castrate controls; here the difference was also greater for males. This is the only organ in which the difference was maintained in spite of castration. Unfortunately both groups do not have the same postoperative time, but because of this result the rôle played by the adrenals in the atrophy of the thymus can not be disregarded.

Attention is called to the absence of any significative difference in the weight of sexual organs. It may be a matter of animal sensitivity, and this suggestion is supported by the increased weight of ovaries in mice one year after operation.

The rôle played by the hypothalamus on sexual function has been extensively reviewed by Anderson and Haymaker (1948) and Benoit and Assenmacher (1955). Several authors have induced ovulation in female rabbits by electrical stimulation of the hypothalamus (Haterius and Derbyshire 1937, Marke et al. 1946, Harris 1937). It is claimed that the median eminence is the place of the hypothalamus responsible for the control of gonadotrophic secretion through the hypophysis, as has been shown by Dey (1943) in guinea pigs, McCann (1953) in rats, Laqueur et al. (1955) in cats. Bogdanove and Halmi (1953) in rats, and Ganong, Fredrickson and Hume (1954) in dogs, have also found alteration in the gonads following hypothalamic lesions.

The results reported in this paper would seem to support the view that hemidecortication releases the hypothalamus of the rat from some restraining action exerted either by the cerebral cortex or by some subcortical structure, thus determining an increased gonadotrophin secretion. In this connection it is worth while to note that Schreiner and Kling (1954, 1956) by bilateral lesions of the amigdaloid complex in agoutis, lynxes, cats and monkeys, induced a state of chronic hypersexuality that was abolished by castration.

A direct action of hemidecortication on the gonads as suggested in the clinical report of Anderson, Haymaker and Rappaport (1948) can not be ruled out.

Summary

The effect of hemidecortication on the endocrine glands in white rats was studied. The results obtained were submitted to a statistical analysis of covariance. Adrenal glands of female operated rats were heavier than the controls and those of male hemidecorticates were lighter. Thyroid glands were lighter in both male and female operated rats. There was no difference between the hypophysis of males, but the hypophysis of female hemidecorticates were heavier than their controls. Thymus was lighter in hemidecorticate rats of both sexes. No differences were found after castration, except for the thymus. An additional group of

hemidecorticate mice showed heavier ovaries than non-operated controls. The assumption is advanced that hemidecortication releases the hypothalamus of the rat from some restraining action exerted either by the cerebral cortex or by some subcortical structure thus resulting in an increased gonadotrophin secretion from the hypophysis.

The authors wish to thank Professor Bernardo A. Houssay for his suggestions and revision of this work.

References

Anderson, E. and Haymaker, W.: *J. Am. med. Wom. Ass.*, 1948, *3*, 402.
Anderson, E., Haymaker, W. and Rappaport, H.: *J. Am. med. Wom. Ass.*, 1948, *3*, 402-257.
Benoit, J. and Aron, M.: *C. R. Soc. Biol., Paris*, 1931, *108*, 786.
Benoit, J. and Assenmacher, J.: *J. Physiologie*, 1955, *427*, 47.
Bialet Laprida, Z.: *Rev. Soc. argent. Biol.*, 1933, *9*, 245; *C. R. Soc. Biol., Paris*, 1933, *114*, 733.
Bogdanove, E. M. and Halmi, N. S.: *Endocrinology*, 1953, *53*, 274.
Bulliard, H., Delsuc, P. and Moday, I.: *C. R. Soc. Biol., Paris*, 1941, *135*, 1120.
Chiodi, H.: *Rev. Soc. argent. Biol.*, 1938; *14*, 309; *C. R. Soc. Biol., Paris*, 1938, *129*, 1258.
Chiodi, H.: Tesis Fac. Med. Buenos Aires, 1938.
Covian, M. R. and Houssay, H. E. J.: *Circulat. Res.*, 1955, *3*, 459.
Covian, M. R., Hartmann, L. F. and Granel, J. A.: *Rev. Soc. argent. Biol.*, 1953, *29*, 42.
Covian, M. R. and Uranga, J.: *Rev. Soc. argent. Biol.*, 1955, *31*, 256.
Covian, M. R., Migliorini, R. H. and Berezin, A.: *Metabolism*, 1958, *7*, 717.
Dey, F. L.: *Anat. Rec.*, 1943, *87*, 285.
Franck, S.: *C. R. Soc. Biol., Paris*, 1937, *125*, 573.
Ganong, W. F., Fredrickson, D. S. and Hume, D. M.: *J. clin. Endocr.*, 1954, *14*, 773.
Harris, G. W.: *Brit. med. J.*, 1950, *6*, 1537.
Harris, G. W.: *Physiol. Rev.*, 1948, *28*, 139.
Harris, G. W.: *Ciba Foundation Colloquia on Endocrinology*, 1952, *4*, 103.
Harris, G. W.: *Neural control of pituitary gland.* London: Arnold Ed., 1955.
Harris, G. W.: *Proc. Roy. Soc.*, s. B. 1937, *122*, 374.
Haterius, H. O. and Derbyshire, A. J. (Jr.): *Amer. J. Physiol.*, 1937, *119*, 329.
Hemphill, R. E.: *Lancet*, 1944, *2*, 345.
Hohlweg: *Klin. Wochenschr.*, 1934, *13*, 92.
Insua, J. A., Florit, J. M. and Velasco Suarez, C. A. (Personal Communication).
Laqueur, G. L., McCann, S. M., Schreiner, L. H., Rosemberg, E., McK. Riock, M., Anderson, E.: *Endocrinology*, 1955, *57*, 44.
McCann, S. M.: *Amer. J. Physiol.*, 1953, *175*, 13.
Marke, J. E., Sawyer, C. H. and Hollinshead, W. H.: *Endocrinology*, 1946, *38*, 345.
Schreiner, L. and Kling, A.: *Arch. Neurol. Psychiat.*, 1954, *72*, 180.
Schreiner, L. and Kling, A.: *Amer. J. Physiol.*, 1956, *184*, 486.
Starr, P.: *J. Amer. med. Ass.*, 1933, *100*, 144.

ACTION OF TESTOSTERONE PROPIONATE UPON THE GROWTH OF HAIR IN RATS

ALBERTO B. HOUSSAY, RICARDO NALLAR and EVA I. SAURER

(Instituto de Biología y Medicina Experimental, Costa Rica 4185 and Centro de Endocrinología, Godoy Cruz 1221, Buenos Aires, Argentina.)

SEVERAL endocrine influences act upon hair follicles and may alter its normal alternate cycles of activity and quiescence (10). As a consequence, the pattern of hair growth upon a denuded area and the time required to restore a normal hair coat are altered (9, 10, 14).

Gonadectomy does not change (4, 9, 16) or slightly increases (15) the rate of the hair cycles in male or female rats. In mice (9, 11), on the other hand, it produces an intense stimulation of all the hair follicles in rest, the cyclic rhythm disappears and the whole denuded area is rapidly and simultaneously covered by a coat of hair. This same type of accelerated hair growth is produced in both rats and mice by adrenalectomy (1, 3, 4, 9, 13, 16) or by hypophysectomy (10, 14).

Steroid hormones, when administered in sufficient amount, inhibit the growth of the hair in rats and mice. This has been proved with estrogens (1, 5-9, 12, 15, 16) and adrenal corticoids (1, 9, 12, 16).

In previous work we have shown that testosterone propionate inhibits the growth of hair in castrated mice (12) and, when given in high doses, in castrated rats (9). The experiments shown in this paper were made in order to study the characteristics of the action of testosterone propionate and the amount required to produce inhibition of hair growth in normal, castrated, adrenalectomized and hypophysectomized rats.

MATERIAL AND METHODS

Male albino rats, weighing from 100 to 150 g, belonging to the colony of the Institute of Biology and Experimental Medicine, were used. Just before the operation, the hair was clipped with an electric clipper number 0000, over an area extending along the back, from the neck to the base of the tail. The animals were clipped as close to the skin as possible, but neither damage nor irritation of the skin occurred.

The time, in days, required to restore a normal appearing coat of hair after close clipping, in every animal, was used as a criterium of the rate of hair

Received for publication, October 2nd, 1958.

TABLE I

Influence of testosterone propionate upon the growth of hair in gonadectomized rats

Testosterone Propionate (micrograms daily)	Animals			Hormone treatment (days)	Hair growth during treatment		
					Complete		Non complete
	Nº	Sex	Final weight (g)		Animals	M ± S. E. (days)	Animals
0	11	M	261	160	11	67 ± 5.5	0
150	9	M	246	160	6	144.6 ± 5.4	3
300	10	M	266	160	9	128.3 ± 9.1	1
600	10	M	238	160	2	125.5 ± 3.5	8
1000	10	M	260	160	0	—	10

growth. The growth of hair in only some areas, or in the greater part of the denuded area, was considered incomplete hair growth.

Several surgical operations were performed upon the rats according to the experiments, such as: castration, adrenalectomy, or hypophysectomy.

The doses of testosterone propionate and cortisone acetate were mixed with lactose and separated into portions, every one containing the daily dose for each subgroup. These portions were dissolved in normal saline, every day and the daily dose was given in a subcutaneous injection of 0.5 ml of the solution, beginning the day after the operation.

When the experiment was over the rats were killed. A complete autopsy was performed and the anterior prostate, posterior prostate, coagulant glands, seminal vesicles, testicles, pituitary, adrenals, thyroid, thymus, kidneys and liver were removed, weighed, fixed in formalin and prepared for histological

TABLE II

Influence of testosterone propionate upon the growth

Testosterone Propionate (micrograms daily)	Animals		Weight of the organs				
	Nº	Final weight (g)	Anterior prostate	Posterior prostate	Coagulant glands	Seminal vesicles (full)	Pituitary
0	10	260.7 ± 31.7	15 ± 1	17 ± 2	—	49 ± 4	13.7 ± 1
150	9	245.6 ± 5.9	859 ± 68	712 ± 39	227 ± 8	2089 ± 187	9 ± 0.5
300	10	266.4 ± 13.9	682 ± 80	671 ± 60	145 ± 18	1583 ± 83	9.3 ± 0.4
600	10	238.4 ± 13.5	849 ± 46	712 ± 34	204 ± 27	1748 ± 150	10.2 ± 1
1000	10	259.6 ± 20.2	994 ± 44	988 ± 121	315 ± 57	2616 ± 126	10.1 ± 1

study. The completeness of adrenalectomy or hypophysectomy was checked by macroscopic examination, in case of doubt by microscopic examination, and in the hypophysectomized animals that did not receive hormones the weight of the adrenals, thyroid, testicles, prostate and seminal vesicles and the variation of body weight were taken into account.

The rats were weighed before the operations (initial weight) and before they were killed (final weight).

In every experiment a statistical study of the results obtained was made, and the average, cuadratic deviation, and standard error were calculated.

Results

Several groups of experiments were made in which the action of testosterone propionate upon the growth of the hair was successively studied in 1) castrated rats, 2) normal rats, 3) hypophysectomized rats and 4) adrenalectomized rats.

Action of testosterone propionate in castrated rats: In experiment 1, several groups of castrated male rats were injected with daily doses of testosterone propionate during 160 days. The 4 groups injected received 150, 300, 600 and 1000 μg daily and there was a 5th. group of uninjected controls. The results upon the growth of the hair appear in table I.

The cyclic growth of hair of castrated and normal rats was observed in all the groups, that is to say, hair growth was not uniform, but there were centers of growth from which it spreads in waves, symmetricaly and gradually. all over the denuded area. Nevertheless, in all the groups receiving testosterone propionate the hair cycles were slower and the time required to recover the denuded area was longer. In some animals, it did not take place during the 160 days following the experiment.

None of the 10 rats receiving 1000 μg daily recovered a complete coat of hair after 160 days. Some of the rats receiving 600 and 1000 μg daily presented complete inhibition of the hair growth similar to that observed in rats receiving high doses of estrogens.

In table II, the weight of the organs of these rats is presented. In all the

II

of hair in gonadectomized rats (weight of the organs)

(mg) M ± S. E.

Adrenals	Thyroid	Thymus	Liver	Heart	Kidneys	Spleen
42 ± 2	29 ± 2	386 ± 30	9552 ± 541	1021 ± 25	2142 ± 30	640 ± 69
37 ± 1	30 ± 2	251 ± 18	10367 ± 459	1073 ± 61	2462 ± 83	547 ± 38
41 ± 3	36 ± 2	286 ± 24	11167 ± 330	1109 ± 63	2570 ± 83	631 ± 59
46 ± 3	30 ± 3	245 ± 12	11522 ± 645	1189 ± 79	2602 ± 74	1047 ± 130
45 ± 2	27 ± 3	317 ± 25	11654 ± 860	1118 ± 40	3100 ± 155	529 ± 24

TABLE III

Influence of testosterone propionate upon the growth of hair in normal rats

Testosterone Propionate (micrograms daily)	Animals			Hormone treatment days	Time in days to restore normal hair coat M ± S. E.
	Nº	Sex	Final weight (g)		
0	10	M	254	150	95.2 ± 10.4
125	9	M	251	150	100.2 ± 4.6
300	10	M	223	150	104.8 ± 3.4

groups there was an intense androgenic action with great increase in the weight of the anterior prostate, posterior prostate, coagulant glands and seminal vesicles, nearly twice as big as those of normal rats of the same weight. Testosterone propionate also produced decrease in weight of pituitary and thymus, increase of weight of liver and kidneys and no significant difference with control rats in the weight of adrenals, thyroid, heart and spleen.

Action of testosterone propionate on normal rats: In experiment 2 we tried to see whether the inhibitory action of the testosterone propionate occurred also in normal rats. 2 groups were respectively given 125 and 300 μg daily for 150 days and compared to one group of uninjected controls.

Table III shows that testosterone propionate at these doses did not inhibit hair growth, the normal cyclic rythm was observed in all the rats and there was no significant difference in the average time for complete hair growth in the different groups.

Table IV shows that these dosages produced a very small and not significant increase of the prostate and seminal vesicles compared to those of the controls which explains its not influencing the growth of the hair. Yet, there was a decrease in the weight of testicles and pituitary.

TABLE IV

Influence of testosterone propionate upon the growth

Testosterone Propionate (micrograms daily)	Animals		Weigh of the organs				
	Nº	Final weight (g)	Anterior prostate	Posterior prostate	Coagulant glands	Seminal vesicles (full)	Testicles
0	10	254 ± 13	377 ± 25	807 ± 21	113 ± 8	1089 ± 59	2887 ± 102
125	9	251 ± 14	438 ± 34	424 ± 27	117 ± 12	994 ± 83	1847 ± 217
300	10	223 ± 8	465 ± 117	394 ± 70	139 ± 59	1309 ± 234	1427 ± 272

TABLE V

Influence of testosterone propionate upon the growth of hair in normal and castrated rats

Testosterone Propionate (micrograms daily)	Animals				Hormone treatment (days)	Gonadectomy	Hair growth during treatment		
	Nº	Sex	Weight (g) Initial	Weight (g) Final			Complete: Animals	Complete: M ± S. E. (days)	Non complete
Experiment 3									
0	9	M	131	310	105	no	9	55.2 ± 5.2	0
1000	3	M	115	208	105	no	0	—	3
0	8	M	127	267	105	yes	8	43.1 ± 6.2	0
1000	6	M	128	202	105	yes	2	77 ± 27	4
Experiment 4							**Time in days to restore normal hair coat M ± S. E.**		
300	9	M	127	313	150	no	59.9 ± 5.7		
300	10	M	119	284	150	yes	79.9 ± 10.2		

As these experiments seemed to show lack of inhibition in normal rats receiving doses that inhibited the hair growth in castrated rats and besides, there was a great difference between the weight of the prostates and seminal vesicles in both experiments, new comparative studies were made, in groups of normal and castrated rats receiving the same daily dose of testosterone propionate, injecting them at the same time and using the same solution.

IV

of hair in normal rats (weight of the organs)

(mg) M ± S. E.

Pituitary	Adrenals	Thyroid	Thymus	Liver	Heart	Kidneys	Spleen
10.8 ± 1	39 ± 2	36 ± 3	198 ± 26	13328 ± 904	874 ± 31	2479 ± 118	515 ± 29
9.1 ± 1	36 ± 2	33 ± 5	294 ± 42	11408 ± 1027	1009 ± 59	2215 ± 160	655 ± 40
7.9 ± 0.5	32 ± 1	29 ± 1	115 ± 16	8967 ± 785	755 ± 36	2021 ± 292	433 ± 14

In experiment 3, there were 4 groups, 1) uninjected normal controls, 2) normal rats receiving a daily dose of 1000 μg of testosterone propionate for 105 days, 3) uninjected castrated rats and 4) castrated rats receiving 1000 μg daily of testosterone propionate for 105 days. In experiment 4 there were 2 groups: 1) normal rats receiving 300 μg of testosterone propionate daily for 150 days and 2) castrated rats receiving 300 μg of testosterone propionate daily for 150 days.

Table V shows the results of both experiments (3 and 4) on hair growth. On one hand, the dose of 1000 μg daily inhibits the hair growth in normal and castrated rats. The rate of hair growth, in the control castrated rats is slightly quicker than in normal uninjected rats, but the difference is not significant. A 300 μg dose of testosterone propionate daily does not inhibit hair growth in normal rats, but it produces slower hair cycles in castrated rats.

In table VI we can compare the weight of the different organs. There is no difference in the weight of prostate and seminal vesicles between normal and castrated rats receiving the same dose of testosterone propionate. Both doses of testosterone propionate are highly androgenic. In these rats the prostate and the seminal vesicles are twice the size of those of the normal uninjected rats. There is a decrease in the weight of the testicles, hypophysis and thymus, and an increase in the adrenals, kidneys and submaxillary glands. The weight of thyroid, liver and heart is not altered.

By comparing the first 4 experiments we can conclude that sufficient amounts of testosterone propionate inhibit hair growth either in normal or in castrated rats, this inhibition being greater in castrated rats receiving the same doses.

We could produce inhibition in castrated rats, giving doses of 150 and 300 μg

TABLE VI

Influence of testosterone propionate upon the growth of hair

Testosterone Propionate (micrograms daily)	Hormone treatment (days)	Animals			Weight of the organs (mg)					
		Nº	Weight (g)		Anterior prostate	Posterior prostate	Coagulant glands	Seminal vesicles		Test
			Initial	Final				Full	Empty	
Experiment 3										
0	105	9	131 ± 8	319 ± 18	364 ± 62	295 ± 25	124 ± 11	908 ± 82	479 ± 50	2613
1000	105	3	115 ± 6	208 ± 11	633 ± 114	703 ± 99	255 ± 95	2263 ± 127	1266 ± 86	1980
0	105	8	127 ± 8	267 ± 17	12 ± 2	23 ± 4	5 ± 0.7	31 ± 3	31 ± 3	-
1000	105	6	128 ± 7	202 ± 15	542 ± 37	646 ± 5	242 ± 30	2290 ± 489	1445 ± 174	-
Experiment 4										
300	150	5	127 ± 8	267 ± 17	610 ± 70	562 ± 68	213 ± 18	1720 ± 202	684 ± 40	1556
300	150	10	119 ± 5	284 ± 12	670 ± 50	546 ± 24	188 ± 13	1260 ± 20	535 ± 31	-

TABLE VII

Influence of testosterone propionate upon the growth of hair in hypophysectomized rats

Testosterone Propionate (micrograms daily)	Animals				Hormone treatment (days)	Hair growth during treatment		
		Sex	Weight (g)			Complete		Non complete
				Final		Animals	M ± S. E. (days)	
0	8	M	128	123	22	14	14.7 ± 0.25	0
100	14	M	127	129	22	4	16.5 ± 1.7	8
600	12	M	120	121	22	0	—	8

of testosterone propionate daily. In normal rats no inhibition was produced with 300 μg daily but an intense inhibition was noticed in those receiving 1000 μg daily

Action of testosterone propionate upon hypophysectomized rats: We tried to see whether testosterone propionate could inhibit the rapid hair growth of the hypophysectomized rats. In experiment 5 we observed 3 groups 1) non-injected hypophysectomized rats, 2) hypophysectomized rats receiving 100 μg of testosterone propionate daily for 22 days and 3) hypophysectomized rats receiving 600 μg of testosterone propionate daily for 22 days.

The results are shown in table VII. The hair grew at the same rate in all

ABLE VI

wth of hair in normal and castrated rats (weight of the organs)

organs (mg) M ± S. E.

npty	Testicles	Pituitary	Adrenals	Thyroid	Thymus	Liver	Heart	Kidneys	Submaxillary glands
± 50	2613 ± 92	9.6 ± 0.6	43 ± 2	27 ± 2	322 ± 34	12874 ± 298	957 ± 34	2449 ± 130	461 ± 30
± 86	1980 ± 42	8.0 ± 0.8	52 ± 6	28 ± 5	80 ± 12	10733 ± 913	877 ± 67	2790 ± 75	516 ± 12
± 3	—	11.8 ± 0.4	44 ± 3	23 ± 2	413 ± 56	10037 ± 350	814 ± 26	1946 ± 121	410 ± 23
± 174	—	8 ± 0.6	59 ± 3	24 ± 2	97 ± 24	11668 ± 490	998 ± 54	2602 ± 52	493 ± 19
± 40	1556 ± 48	10.2 ± 0.9	40 ± 1	32 ± 4	265 ± 18	12686 ± 828	1052 ± 60	2690 ± 75	—
± 34	—	8.6 ± 0.6	40 ± 3	29 ± 2	244 ± 24	11792 ± 763	992 ± 40	2708 ± 159	—

TABLE VIII

Influence of testosterone propionate upon the growth of hair

Dose (ug/day)	Nº of animals	Body weight (g)				
		Initial	Final	Anterior prostate	Posterior prostate	Coagulant glands
0	14	127.6 ± 1.8	123 ± 0.5	14 ± 1	31 ± 0.6	5.1 ± 0.6
100	12	127 ± 1.7	128.7 ± 1.8	167 ± 12	180 ± 6	65 ± 2
600	8	120.1 ± 5	120.9 ± 4.4	206 ± 24	224 ± 24	102 ± 9

the hypophysectomized controls, hence, the small standard error. All the denuded area was covered simultaneously by a homogeneous coat of hair 14 days after operation and clipping. A dose of 100 μg of testosterone propionate daily inhibited the hair growth in 8 out of 12 rats but a dose of 600 μg daily did it in all the injected rats.

The weights of the organs are shown in table VIII. In it, the androgenic action of testosterone propionate with a great increase in the weight of the prostate and seminal vesicles, is pointed out. The weight of the testicle is lowered with a daily dose of 100 μg but it increases with a daily dose of 600 μg. Both doses produce an increase in the weights of the adrenals and the thyroid, but the weight of the thymus and kidneys diminishes.

The great sensitivity of the hair of hypophysectomized rats to the inhibitory action of testosterone propionate is remarkable, since an intense inhibition is produced with a 100 μg daily dose. This dose is apparently physiological since in 22 days it maintains the prostate and seminal vesicles in the same size as that corresponding to normal rats of the same weight.

Action of testosterone propionate upon adrenalectomized rats: We tried to see whether testosterone propionate could inhibit the rapid hair growth in adrenalectomized rats, which present the same characteristics as that of hypophysectomized rats.

Some preliminary experiments showed lack of inhibition using doses which had produced inhibitory action in hypophysectomized rats. Taking into account the fact that there is adrenal function, though slight, in hypophysectomized rats, we tried to potentiate its action with small doses of cortisone acetate which were non inhibitory when given separately.

Experiment 6 shows the lack of inhibition with a daily dose of testosterone propionate of 300 μg for 25 days and with a daily dose of cortisone acetate of 50 and 100 μg for 25 days. But the simultaneous administration of testosterone propionate (300 μg daily) and cortisone acetate (100 μg daily) inhibited hair growth in 2 out of 7 rats.

Experiment 7 shows that testosterone propionate (1000 μg daily) inhibits 3 out of 8 rats, that cortisone acetate (100 μg daily) inhibits 5 out of 9 rats, while the simultaneous administration of both doses of hormones inhibits the 10 injected rats.

In experiment 8 we noticed that the inhibition produced by testosterone

ABLE VIII

owth of hair in hypophysectomized rats (weight of the organs)

Seminal vesicles (full)	Testicles	Adrenals	Thyroid	Thymus	Liver	Kidneys
45 ± 4	826 ± 108	12.7 ± 1	8.5 ± 0.2	—	5971 ± 210	1339 ± 123
521 ± 53	671 ± 315	14.2 ± 1	9.0 ± 0.7	128 ± 16	5259 ± 118	995 ± 58
901 ± 62	1046 ± 41	16.2 ± 1	10.4 ± 1.1	68 ± 17	5490 ± 243	1046 ± 41

TABLE IX

Potentiation of the action of testosterone propionate upon the growth of hair in adrenalectomized rats, by cortisone acetate

Testosterone Propionate (micrograms daily)	Cortisone acetate (micrograms daily)	Animals			Hormone treatment (days)	Hair growth during treatment		
						Complete		Non complete
		Nº	Sex	Final weight (g)		Animals	M ± S. E. (days)	
Experiment 6								
0	0	9	M	178	25	9	14.4 ± 0.5	0
300	0	11	M	140	25	11	16 ± 0.7	0
0	50	6	M	132	25	6	17 ± 0.4	0
0	100	11	M	151	25	11	17 ± 0.6	0
300	50	3	M	152	25	3	17 ± 3.2	0
300	100	7	M	124	25	5	18.8 ± 0.7	2
Experiment 7								
0	0	5	M	201	25	4	16.7 ± 1.8	1
1000	0	8	M	178	25	5	15 ± 0	3
0	100	9	M	199	25	4	20 ± 1.8	5
1000	100	10	M	196	25	0	—	10
Experiment 8								
0	0	10	M	144	25	9	14.4 ± 0.3	1
1000	0	9	M	148	25	0	—	9
0	100	4	M	157	25	3	17.3 ± 1.8	1
1000	100	10	M	167	25	1	11	9

TABLE X

Potentiation of the action of testosterone propionate upon the growth of

Testosterone Propionate (micrograms daily)	Cortisone Acetate (micrograms daily)	Number of animals	Final weight (g)	Weight of the organs		
				Anterior prostate	Posterior prostate	Coagulant glands
Experiment 7						
0	0	5	200.8 ± 12.3	332 ± 43	343 ± 37	123 ± 9
1000	0	8	178.4 ± 8	397 ± 14	465 ± 38	171 ± 17
0	100	9	198.6 ± 7.5	314 ± 30	378 ± 35	140 ± 34
1000	100	10	195.6 ± 13.3	515 ± 28	519 ± 41	188 ± 11
Experiment 8						
0	0	9	191.9 ± 8	241 ± 2.6	259 ± 29	95 ± 10
1000	0	9	148.1 ± 8.6	333 ± 18	384 ± 20	126 ± 7
0	100	4	157.5 ± 6.2	143 ± 12	169 ± 24	71 ± 27
1000	100	10	166.6 ± 8.9	402 ± 1	398 ± 17	123 ± 5

propionate (1000 μg daily) is so intense, since the 9 rats injected are inhibited, that the potentiation cannot be observed.

In table X, the weights of the organs of the animals used in the experiments 7 and 8 are compared. The weights of the prostates and seminal vesicles are higher in the groups receiving testosterone. Cortisone acetate in that dose does not potentiate the action of testosterone upon those organs. In the animals receiving testosterone there is a decrease in the size of the testicles and thymus, the weight of the other organs being in proportion to the body weight.

Through this data we can see that in experiment 7 cortisone acetate, (100 μg daily) potentiated the inhibitory action of testosterone propionate (1000 μg daily) upon hair growth without increasing its androgenic action upon prostate and seminal vesicles.

To know the dose of testosterone propionate able to inhibit the growth of hair in adrenalectomized rats, experiment 9 was performed. It is shown in table IX. There it can be seen, that daily doses of 100 μg or 250 μg do not produce inhibition, whereas half of the animals receiving a daily dose of 500 μg or 750 μg for 25 days are inhibited.

If we remember that in hypophysectomized rats inhibition is produced with a daily dose of 100 μg, we can conclude that their hair follicles are five times more sensitive to the testosterone propionate than those of the adrenalectomized rats.

In table XII the weight of the organs of the rats from experiment 9 is shown.

TABLE X

hair in adrenalectomized rats, by cortisone acetate (weight of the organs)

mg) M ± S. E.

Seminal vesicles		Testicles	Pituitary	Thyroid	Thymus	Liver	Kidneys
Empty	Full						
484 ± 50	—	2410 ± 177	6 ± 0.22	27 ± 4	301 ± 46	—	—
697 ± 35	—	2106 ± 163	6.9 ± 0.5	25 ± 2	194 ± 26	—	—
483 ± 27	—	2572 ± 64	8.8 ± 0.5	33 ± 3	322 ± 28	—	—
700 ± 36	—	2237 ± 54	8.2 ± 1.4	21 ± 1.4	146 ± 3	—	—
387 ± 39	635 ± 85	2416 ± 75	8.6 ± 0.5	35 ± 3	365 ± 20	12760 ± 803	2101 ± 81
537 ± 50	1210 ± 138	1799 ± 125	6.1 ± 0.02	22 ± 2	146 ± 14	7779 ± 574	1713 ± 148
202 ± 27	373 ± 79	2140 ± 122	6.8 ± 0.4	30 ± 4	377 ± 64	6837 ± 323	1642 ± 30
580 ± 25	1389 ± 34	1889 ± 53	7.5 ± 2.0	33 ± 2	248 ± 26	9884 ± 522	1927 ± 123

The action upon the prostate and seminal vesicles is slight with a daily dose of 100 μg and intense in the rest of the groups. There is a decrease in the size of the testicles in all the groups receiving testosterone propionate, except in the one receiving 750 μg daily. In this one there is an increase in the weight of the hypophysis. In all the groups there is a decrease in the size of the thymus, this getting smaller according to the intensity of the dose.

TABLE XI

Influence of testosterone propionate upon the growth of hair in adrenalectomized rats

Testosterone Propionate (micrograms daily)	Animals			Hormone treatment (days)	Hair growth during treatment		
	Nº	Sex	Final weight (g)		Yes	M ± S. E. (days)	Non complete
0	10	M	144	23	9	14.4 ± 0.8	1
100	10	M	164	23	9	16.6 ± 2.3	1
250	10	M	124	23	9	16 ± 0.9	1
500	11	M	133	23	6	16 ± 1.2	5
750	11	M	162	23	5	16.6 ± 0.6	6

TABLE XII

Influence of testosterone propionate upon the growth of ha

Testosterone Propionate (micrograms daily)	Number of animals	Final weight (g)	Weight of the organs (mg)				
			Anterior prostate	Posterior prostate	Coagulant glands	Seminal vesicles	
						Empty	Full
0	10	144 ± 11	159 ± 8	166 ± 11	47 ± 3	182 ± 24	270 ± 65
100	10	164 ± 23	154 ± 33	165 ± 18	52 ± 2	256 ± 48	386 ± 33
250	10	124 ± 8	233 ± 29	231 ± 14	101 ± 7	382 ± 22	796 ± 39
500	11	133.1 ± 6	192 ± 8	337 ± 53	161 ± 6	725 ± 53	1112 ± 39
750	11	162.3 ± 9	374 ± 25	440 ± 16	250 ± 10	—	1655 ± 103

Discussion

Our experiments clearly show the inhibitory action of testosterone propionate upon hair follicles in normal, castrated, adrenalectomized or hypophysectomized rats provided a sufficiently high dose is administered.

The inhibitory action of testosterone propionate was proved for the first time by Houssay and Higgins (1949) in gonadectomized mice (12). Later on, they showed that in castrated mice bearing androgenic adrenal tumours the hair growth was slower than in those clipped immediately after castration (13).

In 1953 we showed the inhibitory action of testosterone propionate in high dosis in castrated rats (9). Notwithstanding these findings work presented by other authors either before or after this date, supports the idea that testosterone propionate has no inhibitory action upon the hair growth in the rat (5-7, 16). In the work of Johnson, 1958 (15) it is shown a slight inhibitory action in castrated rats which decreases the speed rate of the hair cycle down to the one of normal rats, but not beyond.

The difference in the results is partly due to the methods used to measure the hair growth or to the doses given and the time during which they were administered. Thus, Forbes watched the effects upon the coat of hair of the unclipped animal and Emmens measured the length of individual hairs. The highest doses administered by Emmens were 250 μg daily and those given by Mohn, 200 μg daily. Forbes and Johnson used subcutaneous pellets of 7 or 8 mg daily which were slowly reabsorbed.

In our experiment we measured the time required to restore an entirely normal hair coat on a clipped back. This is principally affected by the duration of the stage of quiescence between 2 hair cycles since the duration of the stage of activity varies much less.

All the hormonal factors that inhibit the hair follicles delay the apparition of the next hair cycle, that is to say they prolong the stage of quiescence of the follicles. Thyroidectomy, administration of estrogens, testosterone or adrenal corticoids produce this effect.

ABLE XII

growth *of hair in adrenalectomized rats (weigh of the organs)*

organ (mg) M ± S. E.

icles ull	Testicles	Pituitary	Thyroid	Thymus	Liver	Kidneys
± 65	2210 ± 70	6.6 ± 0.8	20 ± 2	310 ± 17	7715 ± 89	1418 ± 30
± 33	1441 ± 183	7.1 ± 0.7	23 ± 2	261 ± 4	9454 ± 826	1563 ± 81
± 39	1212 ± 128	7.8 ± 0.8	20 ± 2	221 ± 16	6724 ± 464	1231 ± 62
± 39	1812 ± 85	6.4 ± 0.5	20 ± 1	187 ± 9	7373 ± 448	1418 ± 92
± 103	2135 ± 53	10.6 ± 1.2	22 ± 1	165 ± 12	10351 ± 328	1895 ± 87

On the other hand, all the hormonal factors that stimulate the hair follicles, diminish the duration of the stage of quiescence and the next hair cycle appears more quickly. The maximal stimulation appears in gonadectomized mice and in either adrenalectomized or hypophysectomized rats and mice, the stimulus being so intense that, after the operation, all the hair follicles in rest grow at the same time.

Normal and castrated rats present cyclic hair growth. The cycles are slightly shorter in castrated rats. Besides, testosterone propionate inhibits hair growth in both, but castrated rats require smaller doses. The dose to inhibit hair growth, either in normal or in castrated rats, enlarges the prostate and seminal vesicles to twice the size of the normal non-injected rats of the same weight, that is to say, that doses are greater than physiological ones. The doses that diminish the size of the hypophysis, thymus, and testicles are smaller than those that inhibit the hair follicles.

Hypophysectomized rats are the most sensitive to the inhibitory action of testosterone propionate since this action is obtained with a daily dose of 100 μg. The weight of prostate and seminal vesicles, when giving this dose for 22 days, is similar to that of the normal rats of the same weight, then, we can consider it a relatively physiological dose. In fact, in hypophysectomized rats, we are inhibiting hair follicles excited by the operation and can easily understand that this inhibition can be obtained with smaller doses.

In adrenalectomized rats, the hair growth is identical to that of hypophysectomized rats and are much less sensitive to the inhibitory action of testosterone propionate. Only a daily dose of 500 μg can retard the hair growth. This dose produces a considerable enlargement of the prostate and seminal vesicles. Part of the difference in the sensitiveness to testosterone propionate, between hypophysectomized and adrenalectomized animals, is due to the fact that the former maintain the secretion, though diminished, of adrenal corticoids, and these hormones potentiate the inhibitory action of testosterone propionate.

The data of the weight of the organs show that the hair follicles, in white rats, are less sensitive to the action of testosterone propionate than other structures. In other words, higher doses are required for this action than those that

increase the size of the prostate and the seminal vesicles or diminish the weight of the hypophysis, testicle or thymus. Yet, the slight delay in hair growth in normal rats, as compared to castrated rats, shows a slight inhibitory action in physiological doses.

The inhibitory action upon growth of the hair follicle in rats and mice is shared by several steroid hormones such as: adrenal corticoids, estrogens and androgens (9). The adrenal corticoids act in a similar way in both species. Adrenalectomy produces accelerated hair growth which is inhibited by those hormones. This shows that its inhibitory action is physiological and allows the production of normal hair cycles.

On the contrary, there is a difference between the action of estrogenic and androgenic steroids in both species. In male and female mice, castration produces an accelerated hair growth, identical to that of adrenalectomized mice, due to the simultaneous excitation of the hair follicles in quiescence. This shows that in them, androgenic or estrogenic steroids have a physiological inhibitory action which permits the production of normal hair cycles.

On the other hand, in white rats castration produces either little or no changes at all in hair growth, sometimes the hair cycles are a little quicker, which would mean a slight stimulation. This is partly due to the different sensitivity of the hair follicles of both species to these steroids, which has been proved using estradiol benzoate to which the C_3H mouse is 1000 times more sensitive than the white rat (8). This remains still to be proved in the case of testosterone propionate.

It is evident that when a mice is castrated, all the inhibitory factors (probably hormonal factors) are not enough to inhibit the hair follicles which rapidly come into activity. But in the rat, after castration, either a greater quantity of inhibitory factors (probably hormonal factors) remain, or the hair follicles are more sensitive to them, so that they can proceed in a cyclic rhythm. These inhibitory factors may be the adrenal corticoids as it is suggested by the fact that in the rat, only adrenalectomy or hypophysectomy completely suppress such inhibition.

The principal characteristics of the action of testosterone propionate upon the hair growth in the rat, are similar to those of the estradiol benzoate, such as: greater sensitivity of the hypophysectomized rats, less sensitivity of the adrenalectomized rats and potentiation by adrenal corticoids. The action of estradiol benzoate is similar but it requires much smaller doses.

In previous works (9, 14) we have shown that in rats and mice the cycles of hair growth are regulated by hormonal factors. The center of this regulation would be the pituitary gland which would act through two kind of influences: 1) the inhibitory ones produced by adrenocorticotrophin and gonadotrophins and 2) stimulating ones produced by thyrotrophin and perhaps somatrophin. Thus the physiologic action of androgens would be controlled by pituitary gonadotrophins.

Summary

Testosterone propionate, in high doses, produces inhibition in the hair growth of normal, castrated, hypophysectomized or adrenalectomized rats.

The inhibitory action of testosterone propionate upon the hair follicles at the same dosage, is greater in castrated than in normal rats.

The hypophysectomized rats were the most sensitive to this inhibitory action.

Adrenalectomized rats were less sensitive to testosterone propionate; higher doses are required to produce inhibition.

The inhibitory action of testosterone propionate upon hair growth in adrenalectomized rats is potentiated by the simultaneous administration of cortisone acetate.

Bibliografía

(1) Baker, B. L.: *Ann. N.Y. Acad. Sci.*, 1951, *53*, 690.
(2) Butcher, E. D.: *Anat. Rec.*, 1934, *61*, 5.
(3) Butcher, E. D.: *Amer. J. Physiol.*, 1937, *120*, 427.
(4) Diecke, S. H.: *Endocrinology*, 1948, *42*, 315.
(5) Emmens, C. N.: *J. Endocrinol.*, 1942, *3*, 64.
(6) Forbes, T. R.: *Endocrinology* 1942, *30*, 465.
(7) Hooker, C. W., Pfeiffer, C. A.: *Endocrinology*, 1943, *32*, 69.
(8) Houssay, A. B.: *Rev. Soc. argent. Biol.*, 1953, *29*, 221.
(9) Houssay, A. B.: *Acta physiol. lat.-amer.*, 19, 1953, *3*, 232.
(10) Houssay, A. B.: *Ciencia e Invest.*, 1954, *10*, 291.
(11) Houssay, A. B., Higgins, G. M.: *Proc. Mayo Clin.*, 1949, *24*, 269.
(12) Houssay, A. B., Higgins, G. M.: *Proc. Mayo Clin.*, 1949, *24*, 597.
(13) Houssay, A. B., Higgins, G. M.: *Proc. Mayo Clin.*, 1951, *26*, 323.
(14) Houssay, A. B., Penhos, J. C., Foglia, V. G.: *Rev. Soc. argent. Biol.*, 1955, *31*, 7.
(15) Johnson, E.: *J. Endocrinol.*, 1958, *351*, 16.
(16) Mohn, M. P.: *Anat. Rec.*, 1955, *121*, 342.
(17) Mohn, M. P.: *Anat. Rec.*, 1957, *436*, 127.

RELATIONSHIP BETWEEN SERUM CREATININE, ENDOGENOUS CREATININE CLEARANCE AND URINARY CREATININE

CARLOS MONGE C. (*), MANUEL RAMÍREZ V. (**), JAVIER FERNÁNDEZ Ñ. (***) and EDDA HORNA C. (****)

With the technical assistance of Luisa Magán S.

(From the Cátedra de Clínica Médica and the Instituto de Biología Andina, Apartado 821, Lima, Perú.)

INTRODUCTION

THE prediction of creatinine clearance from blood creatinine levels is of theoretical and practical importance. Effersøe (1) has found a prediction error of about ± 20 % in patients with chronic nephropathy in a steady state of creatinine concentration. He considers this error of no clinical importance. We have made a similar study regardless of the steady state or etiology of the nephropathy in order to asses the prediction error in an heterogeneous group of patients with chronic renal failure. We believe our group is more representative of patients generally found in clinical practice.

The data permits us to make a study of the relationship between urinary creatinine and blood creatinine and to emphasize the logarithmic type of function between these two variables.

MATERIALS AND METHODS

Forty male patients who were suspected of renal failure because of their clinical history, physical examination and routine laboratory tests were used in this study. Renal failure was confirmed in the majority of them but for the sake of completeness we also included those cases in whom a laboratory diagnosis was not confirmed.

The endogenous creatinine clearance was determined in two separate periods of one hour each with the exception of two patients in one of whom a single

(*) M. D., Assistant Professor of Medicine, Cátedra de Clínica Médica, Hospital Arzobispo Loayza. Member of the Instituto de Biología Andina. Assistant. Departamento de Medicina, Instituto Nacional de Enfermedades Neoplásicas, Lima.

(**) Member of the Instituto de Biología Andina.

(***) M. D. Instructor of Medicine, Cátedra de Clínica Médica, Hospital Arzobispo Loayza. Member of the Instituto de Biología Andina.

(****) Facultad de Medicina de Lima.

Received for publication, July 8th, 1958.

TABLE I

Plasma and urinary findings in 40 male patients with renal failure

	Age	Weight kg	Surface area m^2	Serum creatinine mg %	Clearance cc/min	Clearance (average) cc/min	Clearance cc/min/1.73 m^2
O. L.	58			1.4	77 - 77	77	
R. Z. L.	35			0.8	121 - 101	111	
O. G. H.	21			1.1	93 - 81	87	
V. W.	49			1.1	74 - 69	71	
E. Z.	18			0.8	127 - 128	127	
J. R. F.	43			1.4	79 - 74	76	
J. P. R.	67			0.8	107 - 108	107	
P. C.	24			1.1	68 - 68	68	
E. O.	33			1.2	110 - 118	114	
J. C.	24			1.2	105 (*) -	105 (*)	
P. A.	46			1.7	83 - 75	79	
J. S.	44			3.1	30 - 38	34	
J. G.	54			1.5	109 - 113	111	
L. B.	61			15.0	3 (**) -	3 (**)	
C. F.	58			1.4	114 - 113	113	
V. C.	48			9.0	10 -	10	
E. M.	70	60	1.70	5.9	14 - 16	15	15
K. H.	51	51	1.49	6.2	9 - 9	9	10
M. V. O. ...	51	54	1.58	0.8	58 - 54	56	61
S. R. M. ...	15	30	1.14	1.1	41 - 44	42	64
N. D.	54	60	1.67	3.1	37 - 31	34	35
H. C.	38	52	1.53	8.4	9 - 9	9	10
C. S.	33	63	1.66	27.4	1 - 1	1	1
S. Z.	46	50	1.54	18.5	3 - 3	3	3
A. M.	42	60	1.60	23.5	3 - 3	3	3
J. E. E.	22	44	1.40	26.3	4 - 4	4	5
G. R. L.	54	67	1.66	7.0	3 - 3	3	3
M. S. Y.	51	74	1.85	2.6	30 (*) -	30	28
J. S.	54	65	1.69	1.7	51 - 48	49	50
R. A.	43	65	1.67	3.2	21 - 21	21	22
D. M.	66	38	1.34	2.8	25 - 18	21	27
A. O. C.	60	66	1.66	1.4	58 - 57	57	59
A. C.	37	64	1.75	8.1	13 - 13	13	13
I. M.	70	66	1.66	10.3	4 - 6	5	5
A. Ch.	58	50	1.46	4.5	15 - 14	14	17
F. G.	21	52	1.48	19.8	6 - 6	6	7
W. K. Y. ...	58	45	1.45	5.2	9 - 9	9	11
M. M.	67	70	1.78	16.9	3 - 3	3	3
L. O. S.	53	63	1.65	13.8	4 - 4	4	4
Ch.	35	68	1.76	13.8	5 - 4	4	4

(*) 12 hours urine collection.
(**) 24 hours urine collection.

collection was done in 12 hours and in another where a single collection was done in 24 hours. The average clearance of the duplicate periods was used in the calculations.

The blood and urine creatinine was determined according to Folin's method as modified by Mandel (3).

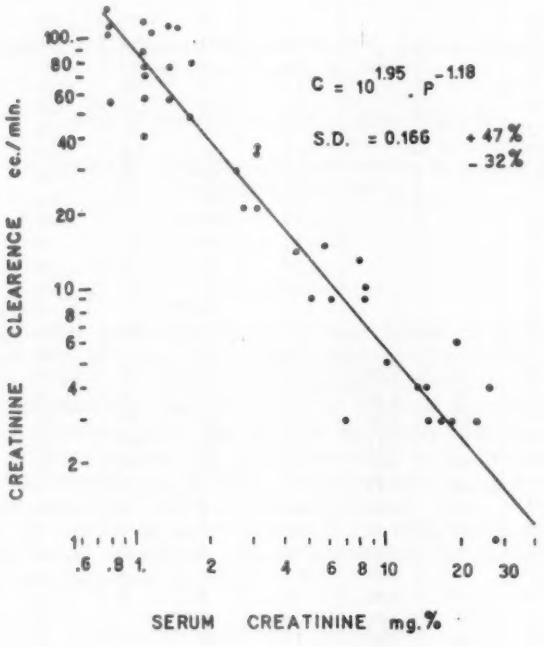


FIG. 1.

RESULTS

The following symbols will be used:

C: Endogenous creatinine clearance expressed in cc/minute.
P: Serum creatinine concentration expressed in mg/100 cc.
UV: Urinary creatinine excretion expressed in mg/minute.

Table I is a summary of the results. Fig. 1 shows the correlation between C and P. The regression equation, $C = 10^{1.95} \cdot P^{-1.18}$ was calculated by the method

of least squares. The standard deviation of this equation is 0.166 which when expressed as a percentage of

$$\text{C is } \begin{matrix} +47\,\% \\ -32\,\% \end{matrix}$$

We may then write:

$$C = \frac{UV}{P} = 10^{1.95} \cdot P^{-1.18}$$

$$\text{or, } UV = 10^{1.95} \cdot P^{-0.18}$$

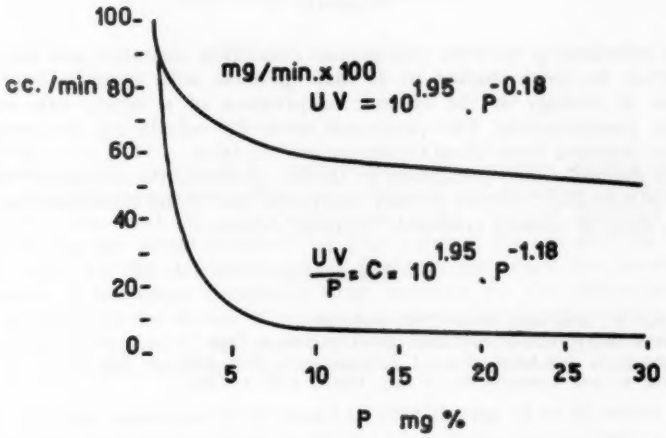


FIG. 2.

This equation indicates that UV is a logarithmic function of P. Fig. 2 shows C as a function of P and UV as a function of P. It can be seen that there is an early drop in the value of UV which later levels off and becomes almost linear.

In 24 cases it was possible to correct for the body surface area by a factor of 1.73 sq. meters. The standard deviation in this group

$$\text{was } \begin{matrix} +54\,\% \\ -35\,\% \end{matrix}$$

DISCUSSION

As can be seen from the results, the prediction error for this group varies from + 47 % to — 32 % and correcting for body surface area did not diminish it. Such an error precludes the use of this method as an estimate of the endogenous creatinine clearance. We suggest that for an heterogeneous group, such as is found in a general hospital, the determination of the endogenous creatinine clearance must be used for assessment of renal function.

It has been shown by Steinitz and Turkand (4), Goldman (2) and Effersøe (1) that UV declines in the course of renal failure. Goldman has used a straight line regression equation to interpret the relationship between UV and P, but as we have shown this relationship is logarithmic and therefore his results are not accurately interpreted from the statistical point of view. Fig. 2 shows the logarithmic relationship between UV and P and it is important to note that UV falls early in renal failure and later descends slowly as an almost linear function of P. At present we are unable to offer an explanation for this type of functional relationship and work is in progress in this laboratory in order to study other important metabolites using UV as a function of P.

Summary

The relationship between endogenous creatinine clearance and blood creatinine levels has been studied in 40 male patients with chronic renal failure regardless of etiology or the absence or presence of a steady state of blood creatinine concentration. The prediction error for calculating the endogenous creatinine clearance from blood creatinine levels is from + 54 % to — 32 % which precludes the use of this prediction in clinical practice. We emphasize the logarithmic relationship between urinary creatinine and blood creatinine levels and the early drop of urinary creatinine in renal failure.

References

(1) Effersøe, P.: *Acta med. scand.* 1957, *156*, 429.
(2) Goldman, R.: *Proc. Soc. exp. Biol.* (N. Y.), 1954, *85*, 446.
(3) Mandel, E. E. and Jones, F. L.: *J. Lab. and clin. Med.* 1953, *41*, 323.
(4) Steinitz, K. and Turkand, H.: *J. clin. Invest.* 1940, *19*, 285.

"CENTRAL" BLOOD VOLUME IN EXERCISE IN NORMAL SUBJECTS

AQUILES J. RONCORONI, PEDRO ARAMENDIA, ROBERTO GONZÁLEZ and ALBERTO C. TAQUINI (*)

THE modifications produced by exercise in cardiac output, oxygen consumption and pulmonary ventilation have received the attention of many investigators (1-4). Less work has been dedicated to the changes produced therein in so called "central" blood volume (5-6). This is due to the fact that the methods currently used for the determination of flow, do not allow the simultaneous measurement of the mean circulation time, necessary for the estimation of the volume of blood in the thorax. Revival of interest in indicator dilution curves as introduced by Stewart (7) and modified by Hamilton (8) has resulted in several studies of "central", "thoracic" or "needle to needle" blood volume, mostly at rest.

Newman and coworkers (9) proposed in 1951 the use of an equation relating the falling dye concentration slope to the cardiac output (Qs) whereby a volume (V) is determined ($V: \frac{Q}{slope}$) which they believed to be representative of the chamber of greater dilution, according to determinations conducted on experimental models.

The object of this study is to estimate the changes produced by exercise in central blood volume as estimated by the Stewart-Hamilton and Newman procedures applied to dye dilution curves.

MATERIAL AND METHODS

Eleven subjects with no evidence of cardiac or respiratory disease were selected from the wards. They were studied after twelve hours fasting and were previously familiarized with the proceeding. An indwelling needle was inserted in the humeral artery of one arm, whilst in the opposite arm, a 35 cm long polyethylene catheter (internal diameter 1 mm) was introduced in an

(*) From the "Centro de Investigaciones Cardiológicas", Azcuénaga 985, University of Buenos Aires, Argentina.

Received for publication, October 4th, 1958.

antecubital vein through a needle, and advanced approximately 30 cm. The subject was then made to stand on a motor driven treadmill.

The ventilation was measured in a Tissot gasmeter by means of a two-way low resistance respiratory valve and appropiate corrugated rubber tubing 4 cm wide. When the ventilation was stabilized, usually after 5 minutes, a 0.5 g %

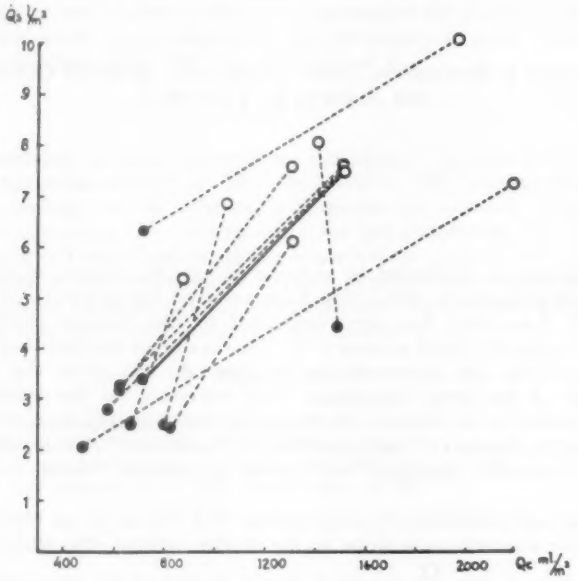


FIG. 1. — *Relationship between cardiac index Qs l/m² in the ordinates and "central" blood volume (Qc ml/m²) in the abscissae. Full circles represent rest conditions, open circles conditions during exercise. The full line connects rest and exercise means.*

solution of Evans blue dye (5 mg for each 10 Kg body weight) was injected intravenously in less than one second through the plastic catheter. Immediately after, serial samples one every two seconds, were collected from the artery in heparinized test tubes. Time intervals were measured with a metronome and the tubes were manually operated.

The amount of dye introduced was determined by weighing of the syringe before and after injection and the amount remaining in the venous catheter estimated by extemporaneous weighing of the same catheter.

Collection of expired air in the gasmeter was started just before the injection and completed in two minutes, gas samples were analyzed for O_2 and CO_2 with the Haldane technique. When the first run of determinations were completed the patient was allowed to sit and ten minutes after the injection, a blood sample was collected (10), for blood volume estimation. The whole

procedure was then repeated five minutes after the patient started to walk in the treadmill at a steady rate (3.5 Km/hour with one degree tilt).

Blood samples were then centrifuged, the plasma was separated and its optical density was measured in a Du Beckman Spectrophotometer at a lengthwave of 6250 Å. The dye concentrations derived were plotted in semilogarithmic paper with extrapolation of the straight descending limb previously to recirculation. Cardiac output was estimated by the formula: $Qs = \frac{I \times 60}{S} / (100 - Ht)$ (8, 9) where I: amount of blue dye injected, S: collected dye concentration every second = surface of curve; Ht: hematocrit. Mean circulation time (M.C.T.) was estimated by Hamilton's (8) equation: $\frac{\Sigma (C.T)}{\Sigma C.}$ were C = dye concentration every second, T: corresponding time; "central" blood volume (Qc) or "needle to needle" volume by Hamilton's (8) modification of Stewart's (7) equation; $Qc = Qs/sec \times M.C.T.$, were Qs: cardiac output, slope volume or Newman's volume was estimated by his formula (10): $\frac{Qs}{slope}$ being $slope = \log K - K_1$, where K and K_1 are respectively, the concentration points corresponding to the beginning of linear logarithmic decay and that one second later.

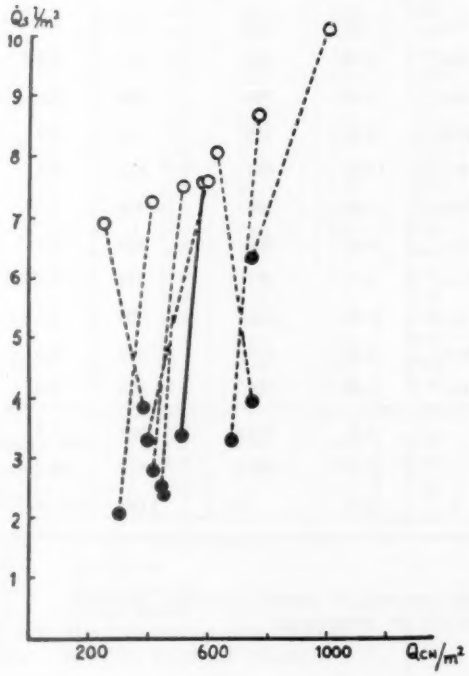


FIG. 2. — *Relationship between cardiac index Qs l/m² in the ordinates and "slope" volume (Qcn ml/m²) in the abscissae, symbols similar to Fig. 1. The full line connects rest and exercise means.*

TABLE I

Respiratory Variables

CASE	NAME	SEX	AGE	B.S.	STATE	VEl/m²	VO_2ml/m²	R.Q.	V.Eq.
1	B.L.	M.	54	1.60	Rest.	5.82	161	.780	3.6
					Ex.	11.88	354	.900	3.3
2	A.R.	M.	49	2.00	Rest.	7.93	182	.786	4.3
					Ex.	19.72	553	.895	3.7
3	B.R.	F.	21	1.50	Rest.	5.96	179	.890	3.3
					Ex.	14.90	416	.940	3.6
4	A.P.	M.	36	1.86	Rest.	9.15	281	.671	3.3
					Ex.	15.10	560	.840	2.7
5	I.P.	M.	48	1.83	Rest.	5.50	138	.794	3.9
					Ex.	15.75	587	.926	2.7
6	I.M.C.	M.	52	1.74	Rest.	5.30	164	.730	2.9
					Ex.	11.20	512	.760	2.2
7	I.M.	M.	24	1.76	Rest.	6.19	188	.844	3.3
					Ex.	13.46	546	.910	2.5
8	F.V.	M.	25	1.91	Rest.	4.90	188	.760	2.6
					Ex.	13.40	634	.850	2.1
9	I.L.	M.	28	1.97	Rest.	6.40	198	.890	3.2
					Ex.	16.60	689	.900	2.4
10	G.A.	M.	52	1.95	Rest.	6.85	207	.810	3.3
					Ex.	20.80	840	.725	2.5
11	S.B.	M.	43	1.70	Rest.	5.40	154	.940	3.5
					Ex.	15.30	526	.875	2.9
		Mean (Rest.)				6.31	185.6	.809	3.4
		Mean (Ex.)				15.28	562.5	.873	2.7
		Mean (Ex.) / Mean (Rest.)				2.42	3.03	1.09	.8

B.S.: Body surface.
V.E l/m²: Expired volume liters/m² (body temperature, standard pressure, saturated).
VO_2ml/m²: Oxygen consumption, mililiters/m² (0° temperature).
R.Q.: Respiratory Quotient.
V.Eq.: Ventilation Equivalent for O_2.

The catheter used for injection was assumed to be lying near the superior vena cava.

Results

Results obtained may be appreciated in Tables I and II. Mean resting ventilation was 6.31 l/m², on exercise there was a 2.4 times increase and the mean attained was 15.28 l/m². Resting oxygen consumption mean value was 185.6 ml/m² and during exercise a mean of 562.3 ml/m² was obtained which resulted in a 3.03 times increase.

TABLE II

Hemodynamic Varibles

CASE	STATE	Hct.	Qbl/m²	Qsl/m²	M.C.T.	Qcl/m²	Qcnl/m²	Qc/Qb%	Qcn/Qb%
1	R.	48.5	2.75	2.06	19.76	.680	.302	24.7	11.0
	E.	48.5	2.75 *	7.28	18.05	2.240	.407	79.7	14.8
2	R.	44.8	3.91	4.48	19.95	1.491	.732	32.8	18.7
	E.	44.3	3.75	8.14	10.37	1.406	.623	37.5	16.6
3	R.	44.9	2.53	3.82	15.67	.997	.386	39.5	15.1
	E.	44.9	2.46	6.90	9.97	1.148	.251	46.5	10.2
4+	R.	46.9	2.64	1.97	22.95	.750	.436	28.5	16.5
	E.	47.0	2.75	4.69	15.98	1.250	.594	45.5	21.6
5	R.	41.0	2.57	2.83	16.73	.772	.415	30.5	16.2
	E.	42.0	2.30	7.55	11.57	1.452	.512	63.1	22.7
6	R.	43.0	3.22	2.41	25.42	1.025	.451	31.8	13.9
	E.	43.0	3.50	6.15	12.76	1.310	.472	34.5	13.5
7	R.	47.0	2.71	6.36	14.40	.916	.745	33.7	27.4
	E.	47.0	3.05	10.20	12.71	2.160	.992	70.9	32.6
8	R.	50.0	2.96	3.31	17.86	.870	.678	32.6	22.4
	E.	50.5	2.98	8.75	10.69	1.558	.762	52.0	25.6
9+	R.	48.0	3.08	3.02	16.12	.811	.476	26.3	15.4
	E.	48.0	3.32	4.61	14.41	1.105	.364	33.2	11.0
10	R.	47.0	3.34	3.31	14.93	.822	.399	26.5	11.9
	E.	47.0	3.69	7.64	10.29	1.310	.596	35.5	16.1
11	R.	50.5	2.72	2.52	20.60	.864	.446	31.7	16.5
	E.	50.5	2.73	5.38	11.96	1.073	.571	39.3	21.0
Mean (Rest.)		46.5	2.95	3.46	18.37	.937	.506	32.1	17.0
Mean (Ex.)		46.6	3.02	7.55	12.04	1.517	.576	51.0	19.2
Mean (Rest.)		—	—	2.18	.65	1.62	1.14	1.59	1.13

Qc: "central" blood volume.
Hct.: Hematocrit.
Qb: blood volume.
Qs: cardiac index.
M.C.T.: Mean circulation time (seconds).
Qcn: slope or Newman's volume.

*: Same figure than at rest was used for exercise Qb.
+: In cases 4 and 9: MCT, Qc, Qcn are excluded from mean figures and statistical study (See text).

Exercise induced a slight increment in respiratory quotient. Ventilation equivalent for oxygen decreased on exercise from a mean figure of 3.4 to 2.7 liters.

Mean figures for arterial hematocrit: 46.5 % and blood volume 2.95 l/m² were not changed during exercise.

In cases 4 and 9 the hemodynamic study was excluded from mean figures computed on Table II since correlation of the figures for resting cardiac output

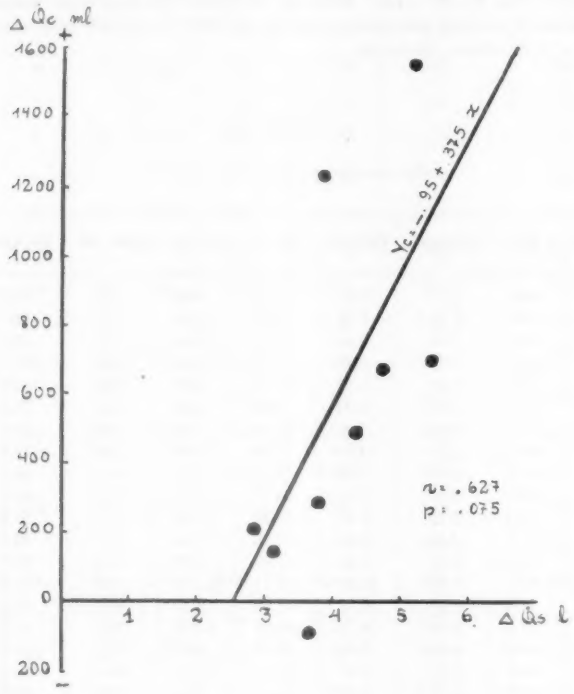


FIG. 3. — *Relationship between changes in cardiac output (Qs exercise — Qs resting) against changes in "central" blood volume (Qc exercise — Qc resting). The trend line fitted by the method of least squares, and its equation are shown.*

in case 4 and exercise cardiac output in case 9 with corresponding oxygen consumptions led us to believe that some mistake had occurred in these determinations.

Cardiac index was 3.46 l/m² (σ : 1.29; σ x: .429) on exercise it was more than doubled reaching 7.55 l/m² (σ : 1.41; σ x : .470); difference between these means was highly significant (p : < .001; t table) (*).

(*) Statistical methods according to Fisher R. A.: Statistical methods for research workers: Oliver and Boyd, Edinburgh, 1938.

TABLE III

Statistical Data

	Qs l/m²		MCT		Qc l/m²		Qcn l/m²	
	Rest.	Ex.	Rest.	Ex.	Rest.	Ex.	Rest.	Ex.
Mean	3.46	7.55	18.37	12.04	.937	1.517	.506	.576
σ	1.29	1.41	3.65	2.47	.257	.458	.392	.212
σ_x	.429	.470	1.22	.82	.085	.152	.131	.076
p	.001		.007		.01		.7	

Similar symbols as in table I and II are used.

σ: standard deviation of the population $\left(\sqrt{\frac{\Sigma x^2}{N-1}}\right)$

p: probability (t table)

σ_x: standard error of the mean $\left(\frac{\sigma}{\sqrt{N}}\right)$

Mean resting circulation time was 18.37 seconds (σ : 3.65; $\tilde{\sigma}$ x : 1.22) and on exercise decreased to 12.04 seconds ($\tilde{\sigma}$: 2.47; σ x : 0.82) difference between means was significant (p : .007). Mean resting "central blood volume" was .937 l/m² (σ : .257; σ x : .085) it reached 1.517 l/m² on exercise (σ : .458; σ x : .152) the difference between means of .580 l/m² was significant (p = .01). Mean slope volume at rest was .506 l/m² (σ : .392 and σ x : .131) and on exercise .576 l/m² (σ : .212; σ x : .076), this difference was not statistically significant (p = .7).

Mean "central" blood volume was 32.1 % of total blood volume and increased to 51 % in exercise. Slope volume was 17 % of total blood volume and changed only slightly during exercise.

Discussion

All subjects hyperventilated while standing at rest if the mean figure of 6.31 l/m² is compared to the usual standards (12). Oxygen consumption was proportionately less increased which resulted in higher than normal ventilation equivalent for oxygen.

The exercise used, which determined a three fold increase in oxygen consumption can be classified as a moderate effort and was well tolerated by all patients. In agreement with data previously reported for normal individuals some decrease in ventilatory equivalent for oxygen was found (12-14).

Mean standing cardiac index fell within the normal range and is very near to the figure obtained by Chapman (15) with the same technique; in evaluating this variant it must be taken into account that it decreases in standing subjects (16).

Exercise produced a little more than a two times increase in flow rate which, when compared with the change in oxygen consumption indicates that arterio-venous O_2 difference must have been elevated at that time. This is in keeping with the results obtained during a moderate exercise by Donald and coworkers (4).

The published figures for mean circulation time (M.C.T.) must be evaluated in relation to the site of injection of the dye. Thus when it was made through a needle placed in an antecubital vein the M.C.T. was 32.1 seconds for Hetzel at al. (17) and the median circulation time 30.1 seconds for Mills and Kattus (18). On the other hand, by injection through a catheter placed in the pulmonary artery Ebert (19) obtained a median circulation time of 10.2 seconds and Doyle (20) 11.5 seconds. Our M.C.T. of 18.37 seconds compares favourably with the figures of Chapman and Fraser (15) of 18.2 seconds. Considerations of the same nature may be attributed to resting "central blood volume" since peripheral injection, through the higher M.C.T., includes the volume of blood contained in all veins reaching the heart at equal mean time. So Hamilton found 1.500 ml/m², Mills and Kattus: 1.700 ml/m², Ebert 1.160 ml total body surface, Doyle 634 ml/m² and Hetzel et al. 1.080 ml/m² by pulmonary injection and 1.830 ml/m² by peripheral vein injection.

Our own mean of 937 ml/m² is placed intermediately as it should be, according to the intermediate injection site and is 32.1 % of the total blood volume.

The slope, or Newman's volume is not influenced, according to Newman (21) by changing the injection point, so our mean of 506 ml/m² is not significatively different from the 530 ml/m² obtained with peripheral vein injection by Mills and Kattus (18). During exercise the mean circulation time decreased in all subjects, the mean value of 12.04 seconds is similar to that of 12.1 seconds presented by Chapman and Fraser (15).

Considerations on the fate of "central" blood volume during exercise must take into account the fact that it is the resultant of two independently determined variables: cardiac output and M.C.T. since ($Qc = Qs/sec. \times M.C.T.$).

If the cardiac output increase during exercise was matched by a similar decrease in M.C.T., central blood volume would not change. As it is, the decrease in M.C.T. showed a variable amount of compensation with variable increase in "central" blood volume. Thus, in case 2, "central" volume did not change, while in cases 1 and 7 it increased tremendously (*); in one of the subjects presented by Chapman (15) (case I.T.), "central" blood volume derived from the tabulated data increased from 2.353 ml to 5.943 ml while Kaufman (6) in two instances, obtained a "central" volume during exercise equal to the total blood volume. In all our other cases the relationship obtained, showed a moderate increase in "central" volume, with a mean overall figure of 1.517 ml/m² which is .62 time more than the resting value and 51 % of total blood volume.

Correlation between cardiac output and "central" blood volume has been sought by several investigators. Doyle et al. (22) related these variables in different subjects and were able to show that in the presence of large flows their changes are not associated whith modifications of "central" volume, whereas when the flows are normal or low any increase is associated with larger "central"

(*) No error was apparent after careful consideration of the dye concentration curve in these cases.

volumes. Sjöstrand (23) believes that blood in the thorax acts as a reservoir of the first order, balancing the changes in venous return and the output of the left ventricle. In spite of that, he could not find by indirect determinations any difference in the vascular capacity of the thorax during exercise (23) in agreement with Asmussen et al. (16). Johnson (24) accepting the same function of the blood in the lesser circuit as a blood reservoir found a positive correlation between decrease in cardiac output and "central" blood volume (measured by dye-dilution) during anesthesia. Identical results have been communicated later by Etsten and Li (25). Rapaport et al. (5) in mitral stenosis found a good positive correlation between blood flow and "central" blood volume which appeared to be maintained on exercise. They accept Johnson's interpretation of the probable need of an increase in the amount of blood in the venous bed of the lungs and left heart in order to produce the augmented filling pressure of the left heart associated with higher outputs.

In our cases, correlation between flow and "central" blood volume was poor at rest ($r = +.370$; $p = .3$) and considerably better during exercise ($r = +.726$; $p = .03$) Comparing changes (fig. 3) between these variables (Qs exercise $-$Qs rest and Qc exercise$-$ Qc rest) the correlation was found to be somewhat decreased ($r = +.627$; $p = .07$).

It may be inferred from fig. 3 that the smaller increases in cardiac output may not be associated with modifications in "central" blood volume. It would appear then, that in those circumstances, flow was augmented and mean circulation time reduced proportionately. Variability of results, at higher output levels, might depend on different combinations of flow increase and M.C.T. decrease. It is also probable that at particular levels of work (perhaps depending on the available cardiac reserve) a higher filling pressure, obtained by "central" blood volume increase, is needed.

Radiological estimation of the heart volume during effort shows it to be unchanged or decreased (26). If the amount of blood contained in the heart and lungs does not increase during exercise, (since "slope volume" does not change) it follows that the only possible sites in wich the blood can accumulate, would be the corresponding systemic and pulmonary veins, accepting that the volume of the arteries involved does not suffer any significant change. This conclusion seems to be sustained by Kaufman (6) by his determination of the lung-ear appearance time, which he found scarcely changed during exercise, and if representative of the same behaviour for the mean time, would mean, in face of an increased "central" volume, that the vascular compartment represented by that "time" i.e. the pulmonary veins, or a segment therein contains a big part of the increase in central blood volume.

In our cases, exercise did not produce a significant change in mean "slope" volume. On the other hand the mean increase in "central" blood volume reached .580 l/m^2, which, if Newman's ideas are literally accepted, must be distributed between the heart, equitemporal veins and arteries.

As there is no determination of the limits of the vascular bed of the lungs included into the "slope" volume, no inference may be drawn regarding the precise place of collection of the extra blood during exercise. We believe that conclusions on this point must await the development of new techniques for the appreciation of the vascular capacities discussed.

Summary and Conclusions

Hemodynamic changes produced by a moderate exercise in standing subjects are presented. During effort, the volume of packed red cells and total blood volume remained the same. Mean "slope" or Newman's volume was not changed. Cardiac output was doubled and "central" blood volume increased variably. Correlation between these last variables, which was poor at rest, increased considerably during exercise. These results are discussed.

References

(1) Riley, R. L., Himmelstein, A., Motley, H. L., Weiner, H. M. and Cournand, A.: Studies of the pulmonary circulation at rest and during exercise in normal individual and in patients whith chronic pulmonary disease. *Amer. J. Physiol.*, 1948, *152*, 372.
(2) Dexter, L., Whittenberger, J. L., Haynes, F. W., Goodale, W. T., Gorlin, R. and Sawyer, C. S.: Effects of exercise on circulatory dynamics of normal individuals. *J. appl. Physiol.*, 1951, *3*, 439.
(3) Asmussen, E. and Nielsen, M.: The cardiac output in rest and work determined simultaneously by the acetylene and the dye injection methods. *Acta physiol. scand.*, 1952, *27*, 217.
(4) Donald, K. W., Bishop, J. M., Cumming, G. and Wade, O. L.: The effect of exercise on the cardiac output and circulatory dynamics of normal subjects. *Clin. Sci.*, 1955, *14*, 37.
(5) Rappaport, E., Kuida, H., Haynes, F. H. and Dexter, L.: The pulmonary blood volume in mitral stenosis. *J. clin. Invest.*, 1956, *35*, 1393.
(6) Kaufman, G.: Uber Kreislanfzeiten und Blutverteilung bei Arbeit. *Cardiologia*, 1957, *30*, 102.
(7) Stewart, G. N.: The pulmonary circulation time, the quantity of blood in the lungs and the output of the heart. *Amer. J. Physiol.*, 1921, *58*, 20.
(8) Hamilton, W. F., Moore, J. W., Kinsman, J. M. and Spurling, R. G.: Studies on the circulation. I: Injection method. Physical and mathematical considerations. *Amer. J. Physiol.*, 1929, *89*, 322.
(9) Hamilton, W. F., Moore, J. W., Kinsman, J. M. and Spurling, R. G.: Studies on the circulation. IV: Further analysis of the injection method, and of changes in hemodynamics under physiological and pathological conditions. *Amer. J. Physiol.*, 1932, *99*, 534.
(10) Newman, E. V., Merrel, M., Genecin, A., Monge, A., Milnor, W. R. and Mc Keever, W. P.: The dye dilution method for describing the central circulation. An analysis of factors shaping the time-concentration curves. *Circulation*, 1951, *4*, 735.
(11) Noble, R. F. and Gregersen, M.: Mixing time and disappearance rate of T-1824 in normal subjects and in patients in shock. Determination of plasma volume in man from 10 minutes sample. *J. clin. Invest.*, 1946, *25*, 158.
(12) Cournand, A. and Richards, D. W. (Jr.): Pulmonary insufficiency. I: Discussion of a physiological classification and presentation of clinical tests. *Amer. Rev. Tuberc.*, 1941, *44*, 26.
(13) Anthony, A. J.: Untersuchungen über Lungenvolumina und Lungenventilation. *Deutsches Arch. f. klin. Med.*, 1930, *167*, 129.
(14) Comroe, J. H. (Jr.): Interpretation of commonly used pulmonary function tests. *Amer. J. Med.*, 1951, *10*, 356.
(15) Chapman, C. B. and Fraser, R. S.: Studies on the effect of exercise on cardiovascular function. I: Cardiac output and mean circulation time. *Circulation*, 1954, *9*, 57.
(16) Asmussen, E. and Nielsen, M.: Cardiac output during muscular work and its regulation. *Physiol. Rev.*, 1955, *35*, 778.
(17) Hetzel, P. S., Swan, H. J. C. and Wood, E. H.: Influence of injection site on arterial dilution curves of T-1824. *J. appl. Physiol.*, 1954, *7*, 66.
(18) Mills, H. and Kattus, A. A.: A comparison of volumes calculated from median circulation time and from slope of human dye dilution curves. *J. lab. Clin. Med.*, 1956, *48*, 413.
(19) Ebert, R. V., Borden, C. W., Wells, H. S. and Wilson, R. N.: Studies of the pulmonary circulation. I: The circulation time from the pulmonary artery to the femoral artery and

the quantity of blood in the lungs in normal individuals. *J. clin. Invest.*, 1949, *28*, 1134.
(20) DOYLE, J. T., WILSON, J. S., LEPINE, C. and WARREN, J. V.: An evaluation of the measurement of the cardiac output and of the so called pulmonary blood volume by the dye-dilution method. *J. lab. Clin. Med.*, 1953, *41*, 29.
(21) PEARCE, M. L., MC. KEEVER, W. P., DOW, P. and NEWMAN, E. V.: The influence of injection site upon the form of dye-dilution curves. *Circulat. Res.*, 1953, *1*, 112.
(22) DOYLE, J. T., LEE, R. A., KELLEY, E. B.: Observations on the elasticity of the pulmonary vasculature in man. *Amer. Heart J.*, 1952, *44*, 565.
(23) SJÖSTRAND, T.: Volume and distribution of blood and their significance in regulating the circulation. *Physiol. Rev.*, 1953, *33*, 202.
(24) JOHNSON, S. R.: The effect of some anaesthetic agents on the circulation in man. *Acta chirurg. scand.*, 1951, suppl. 158.
(25) ETSTEN, B. and LI T.: Hemodynamic changes during thiopental anesthesia in humans, cardiac output, stroke volume, total peripheral resistance and intrathoracic blood volume. *J. clin. Invest.*, 1953, *34*, 500.
(26) KJELLBERG, S. R., RUDHE, U. and SJÖSTRAND, T.: The amount of hemoglobin (blood volume) in relation to the pulse rate and heart volume during work. *Acta physiol. scand.*, 1950, *19*, 152.

EFFECT OF BUTYRATE ON VENTRICULAR FIBRILLATION INDUCED BY LACK OF METABOLITES ON THE ISOLATED GUINEA PIG HEART

MARIO PENNA, ALEJANDRO ILLANES, JAIME RUIZ and JORGE MARDONES

(Instituto de Farmacologia, Escuela de Medicina, Universidad de Chile.)

IN A PREVIOUS paper (1) we have reported that isolated guinea pig hearts when perfused according to Langendorff's technique with a substrate-free Tyrode solution progressively showed decrease in rate, ventricular premature contractions, A-V dissociation, decrease in the voltage of the complexes and finally ventricular fibrillation. This last rhythm disorder was reversed to normal or idioventricular rhythm when glucose was added to the perfusing fluid.

Since cardiac muscle is able to utilize energy from fat metabolites and it is able to convert C^{14} labelled butyrate to pyruvate and lactate (2), it was interesting to study if butyrate is able to reverse ventricular fibrillation induced by lack of metabolites in the perfusion fluid.

METHOD

The method has been described previously (1). Experiments were performed on isolated guinea pig hearts perfused with oxygenated Tyrode solution, according to Langendorff's technique. A special device allowed to change the perfusing solutions keeping constant temperature, pressure and oxygenation.

The electrogram was registered in a Model III D Grass Electroencephalograph. Three bipolar leads were taken simultaneously and the electrogram studied in the same way as described (1).

RESULTS

Eight isolated guinea pig hearts were perfused with glucose-free Tyrode solution. Three to four minutes after ventricular fibrillation was established the perfusing solution was shifted to Tyrode solution without glucose containing butyrate (Butyric acid, Merck, Darmstadt 0,25 ml/l). Table 1 summarizes the experimental results. In order to simplify the data we have included only the initial state and that at 14 minutes before the beginning of ventricular fibrillation as well as data recorded every 15 minutes after the addition of butyrate.

Ventricular fibrillation. – The time elapsed between the starting of the per-

Received for publication, April 20th, 1958.

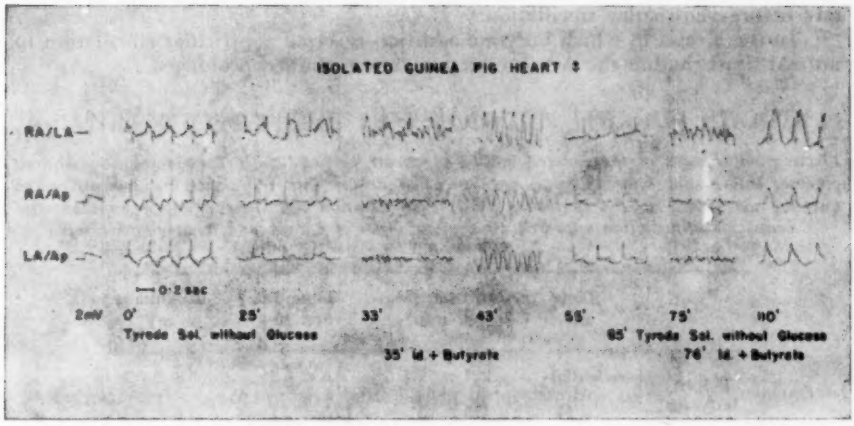


FIG. 1. — *Isolated guinea pig heart perfused with the Langendorff technique. RA/LA lead right auricle/left auricle. RA/Ap lead right auricle/apex. LA/Ap lead left auricle/apex. (Time in minutes after starting the experiment is shown at the bottom.)*

fusion without metabolite and the beginning of ventricular fibrillation ranged from 31 to 120 minutes. The evolution of the electrogram changes during this period was similar to those previously reported (1).

In all the hearts under ventricular fibrillation the perfusion with Tyrode solution containing butyrate and no glucose stopped it and ventricular complexes reappeared in a lapse of about 15 minutes. In 5 cases a normal sinus rhythm was reinstalled and in the 3 others a ventricular rhythm appeared. Preceeding the normal rhythm, ventricular flutter and ventricular tachycardia were observed as intermediate states.

In two cases, one of which is represented in Fig. 1, after ventricular activity was reinstalled, substrate-free Tyrode solution was perfused again. In both cases ventricular fibrillation reappeared and the addition of butyrate reversed the mechanism again to ventricular rhythm.

Auricular rate. — The auricular rate decreased during the perfusion with glucose-free Tyrode solution. During the four minute period of ventricular fibrillation had an average of 77 % of the initial rate. After butyrate addition, auricular rate remained at the same level during the observation time (about 60 minutes).

Voltage of the R wave. — During the perfusion with glucose-free Tyrode solution, the R voltage decreased slowly and at the moment preceeding ventricular fibrillation had an average of 45 % of the initial values. The addition of butyrate did not recover the R voltage and a slight transient decrease was observed initially.

A-V Conduction. — During the perfusion with Tyrode without glucose, lengthening of the A-V conduction was observed. In five cases complete A-V dissociation was registered at ten minutes before starting ventricular fibrillation. In the other three cases at that moment the PR interval was prolonged to nearly

three times the initial values, and complete A-V dissociation appeared immediately before ventricular fibrillation.

In the 5 cases in which butyrate addition reversed ventricular fibrillation to normal sinus rhythm the A-V conduction time remained prolonged.

TABLE I

Electrogram changes of the isolated guinea pig heart perfused with Tyrode solution without glucose, before and after butyrate ardition. The dotted line represents the moment when butyrate was added to the perfusing fluid. This addition was done about 4 minutes after ventricular fibrillation appeared. Arithmetic mean of 8 cases and its standard errors

	Time min.	Auricular rate per min.	Time PR Sec. 10-2	R Voltage mV
Before butyrate	Initial	221 ± 7.4	4.6 ± 0.2	5.8 ± 0.9
	— 14	175 ± 5	12.5 ± 2.3	2.6 ± 0.5
After butyrate	+ 8	181 ± 14.4	—(*)	—(*)
	+ 24	173 ± 8.1	9.8 ± 2.0 (**)	1.4 ± 0.5
	+ 36	171 ± 6.3	10.8 ± 1.1 (**)	1.8 ± 0.6
	+ 52	173 ± 7.5	10.1 ± 0.7 (**)	2.3 ± 0.9

(*) Ventricular activity has not yet recovered.
(**) In the 5 cases without A-V dissociation.

Conclusions

The experimental results show that the guinea pig heart is able to utilize the energy of butyrate for the re-establishment of the rhythmicity disturbed by lack of metabolites in the perfusion fluid, and are consistent with unpublished experiments performed by Garb and Penna (3) showing that butyrate and β-hydroxibutyrate were able to effect a partial recovery of the amplitude of the contractions on isolated rat hearts made hypodynamic by perfusion with substrate-free Ringer-Locke solution.

Summary

Butyrate reverses to normal or ventricular rhythm the ventricular fibrillation induced on the isolated guinea pig heart by perfusion with glucose-free Tyrode solution.

References

(1) Penna, M., Illanes, A., Rivera, J., Mardones, J.: *Acta physiol. lat.-amer.* 1957, *7*, 110.
(2) Lorber, V. quoted by Bing, R. J., Siegel, A., Unger, I., Gilbert, M.: *Amer. J. Med.* 1954, *16*, 504.
(3) Garb, S., Penna, M.: (Unpublished experiments).

ADRENOCORTICAL HORMONE IN HUMAN BLOOD

CARMEN DEL CAMPO DE HACHEN

(Head of Investigations, Pharmacology Division of the Faculty of Medicine, Universidad Nacional del Litoral, Rosario, Argentina.)

SEVERAL methods are now available for the determination of Free and conjugated corticosteroids in blood (1-6).

Several solvents have been used for the extraction of these steroids including chloroform, ethyl acetate and butanol. In human peripheral blood, Bush (7) found the main 17-hydroxycorticosteroid to be cortisol with only traces of corticosterone present; ethyl acetate was used as extraction solvent. Bayliss (5), using chloroform, found that one half to one third of the free 17-hydroxycorticosteroids was cortisol while the remainder consisted of more polar material which appeared to include tetrahydrocortisol. Reddy et al. (6) used butanol for the extraction of the total 17-hydroxycorticosteroids; in the conjugated fraction they found chiefly tetrahydrocortisol.

In the present work the solubility of pure corticosteroids in redistilled n-butanol, ethyl acetate and chloroform are determined as well as their partition coefficients. Recoveries after elution from paper and column are reported as well as the nature of corticosteroids found in blood extracts prepared with butanol. (*)

PROCEDURE

Amounts of 180 ml of blood were obtained from the blood bank. Only blood which had been stored for less than 12 hours was used. Aliquots of 75 ml each were extracted with half the volume of redistilled n-butanol in a centrifuge tube. The contents were thoroughly mixed by stirring by hand for 2 minutes with a glass rod of corkscrew design and were centrifuged for fifteen minutes at 2500 r.p.m. The butanol layer was removed by means of a pipette and a second extraction with butanol was carried out using one fifth the volume of the blood.

The emulsion that remains in the tube can be broken up by stirring with a glass rod, mixed with the whole content and centrifuged; the new layer of butanol that appears can be removed and combined with the previous butanol extracts. Eighty per cent of the n-butanol added could be removed by this procedure.

(*) The following abbreviations have been used: THF (tetrahydrocortisol), THE (tetrahydrocortisone), Compound F (cortisol), Compound E (cortisone).

Received for publication, October 6th, 1958.

Evaporation of Butanol.

The combined butanol extracts were placed in a beaker and the contents were evaporated overnight in the stove at a temperature of 40° C.

When the evaporation was completed, the residue was dissolved in ethyl acetate and conjugated corticosteroids were extracted with three washings of water and discarded, the free fraction remaining in the solvent phase. The ethyl acetate was separated and evaporated. The residue was further purified on a Florisil column prepared according to the method of Nelson and Samuels (1). The column was washed first with 10 ml of chloroform which was discarded, and then the corticosteroids were eluted with 20 ml of a mixture of ethanol and ether (3 : 1).

The alcohol ether extracts obtained from the Florisil column were combined and were evaporated to dryness at 40° C.

Occasionally the butanol extracts of blood ware evaporated over a filter paper in a beaker by suspending vertically in the liquid a strip of Whatman Nº 3 filter paper, 2 cm $\times$ 10 cms and maintained overnight at 40° C (the solids remain in the filter paper after the butanol was completely evaporated). This filter paper was cut out into small pieces which were returned to a beaker and extracted three times for 2 minutes with redistilled ethyl acetate. The conjugated fraction remains in the paper and the free fraction passes into the ethyl acetate.

The final purified ethyl acetate residue was dissolved in a small amount of alcohol-ether solution and applied on a strip of paper (Whatman Nº 1) and developed in the benzene-formamide system according to the method of Zaffaroni (8). Occasionally the benzene-aqueous methanol (55 ml of methanol in 100 ml) system of Bush (2) on Whatman Nº 2 paper was used for comparison.

A pilot strip containing compounds F, E and THE were run simultaneously. The position of the corticosteroids on the paper was determined by means of ultraviolet light absorption and their ability to reduce the tetrazolium blue solution. The method used has been described (9). The areas on the paper showing positive reactions were cut, eluted, and the concentration of the 17-hydroxycorticosteroids determined by the Porter-Silber technique (3). The various steroids were characterized by their Rf values and by mixed chromatograms with authentic compounds. The identity of the cortisol and tetrahydrocortisone in the blood extracts was further established by absorption spectra of the sulfuric acid chromogens in the Beckman spectrophotometer (10).

Results

The corticosteroids which were consistently found in the blood extracts were cortisol and tetrahydrocortisone. Cortisol ranged from 7 to 16.5 μg per 100 ml of blood while tetrahydrocortisone ranged from 12 to 27 μg per 100 ml of blood. Cortisone was present only in small amounts and in some bloods the concentration was too weak to be detected. In a few samples of blood, material more polar than THE which reduced blue tetrazolium was observed. The Rf value corresponded to that of tetrahydrocortisone.

The sulfuric acid spectrum showed maxima at 285, 320, 390 and 470 mμ for cortisol and 410 mμ for tetrahydrocortisone. There was not sufficient material to carry out the reactions of cortisone and tetrahydrocortisol.

TABLE I

Corticosteroids found in peripheral blood

Sample	THF μg %	THE μg %	F μg %	E μg %	Total	F/THE
I	7	12	7	4	30	0.58
II	12	18	10.2	3	43.2	0.56
III	—	22	15	5	42	0.68
IV	—	21	16.5	4	41.5	0.78
V	—	19	12	3	34	0.63

The total amounts of corticosteroids isolated in five samples of blood ranged from 30 to 43.2 μg per 100 ml of blood. The ratio of cortisol to tetrahydrocortisone was relatively constant varying from 0.56 to 0.78. The results are shown in Table 1.

Recovery of Corticosteroids.

Hydrocortisone, cortisone, tetrahydrocortisone and tetrahydrocortisol were dissolved in 0.1 to 0.5 ml of methanol and added to 10 ml of water at 20° C. These solutions were thoroughly mixed with equal volumes of chloroform, ethyl acetate and n-butanol and the recovery of the variuos corticosteroids determined by means of the Porter-Silber reaction. The results are shown in Fig. 1. Chloroform extracted only 2 per cent of cortisol and 3.8 per cent of the cortisone from the aqueous solution. Ethyl acetate was more efficient, extracting 31 per cent of cortisol and 27 per cent of the cortisone. Butanol removed much larger amounts of the steroids; the recovery of cortisol was 100 per cent and that of cortisone 76 per cent. The recovery of THE and THF were 100 per cent and 68 per cent respectively. The distribution coefficient of cortisone in ethyl acetate was 0.37 and that of cortisol 0.45.

The solubility of these four corticosteroids in n-butanol was determined. They are as follows:

STEROIDS	SOLUBILITY mg./ml. at 20° C.
THF	25.0
THE	15.0
F	10.7
E	6.2

The recovery of tetrahydrocortisone from columns of Florisil and silica gel was investigated as these methods were used for the purification of the blood extracts. The Florisil column was prepared according to the method of Nelson

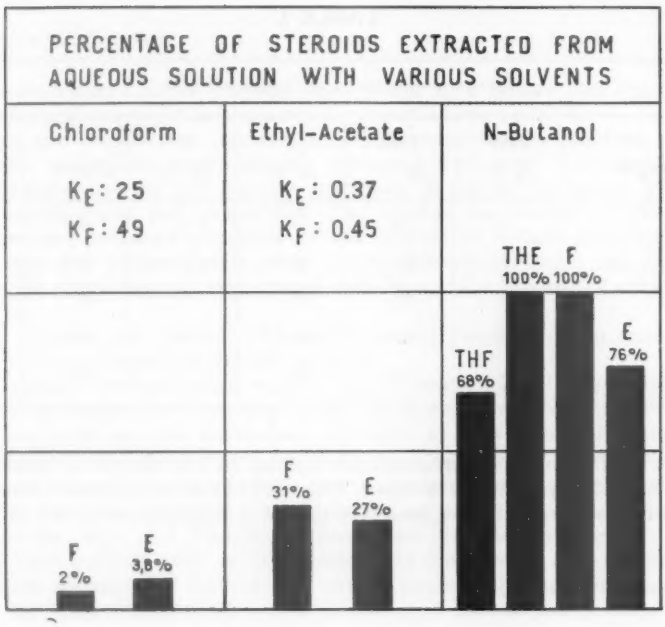


FIG. 1. — K_E: distribution coefficient for *Compound C*. K_F: distribution coefficient for *Compound F*.

and Samuels (1). Sixty μg of tetrahydrocortisone dissolved in chloroform were added and elution from the column was carried out with 10 ml of chloroform and 20 ml of a mixture of ethanol-ether (3 : 1). It was found that under these conditions the chloroform eluted from the column 10 μg of the compound or 16 per cent and the alcohol ether mixture 45 μg. or 75 per cent. A similar experiment was carried out using silica gel. The eluting solvents were benzene and a mixture of alcohol ether (3:1). Only traces of tetrahydrocortisone were eluted from the silica gel with benzene, the alcohol-ether mixture eluted 40.6 μg. or 67 per cent. The results are shown in Table II.

Tetrahydrocortisone dissolved in butanol was applied on strips of Whatman filter paper Nº 3. The paper was dried at 40° C. and were eluted with the various solvents as shown in Table III. Three successive extractions of 4, 2 and 2 ml. were used at intervals of 30 minutes. The results show that the alcohol ether 3:1 mixture was more efficient than benzene or chloroform for the elution of tetrahydrocortisone from the paper.

DISCUSSION

Butanol dissolves greater amounts of pure corticosteroids than ethyl acetate or chloroform. Butanol dissolves in one shaking about 100 per cent of THE

TABLE II

Recovery of tetrahydrocortisone from column

Eluent 25 ml.	FLORISIL			SILICA GEL		
	Added µg	Recoved µg	Recovery %	Added µg	Recoved µg	Recovery %
Benzene	—	—	—	60	Traces	—
Alcohol-Ether 3:1 .	—	—	—	60	40.6	67
Chloroform	60	10	16			
Alcohol-Ether 3-1 .	60	45	75			

present in water, about 76 per cent of compound E, 100 per cent of compound F and 68 per cent of compound F. Ethyl acetate was found to dissolve 31 per cent of compound F and 27 per cent of compound E after one extraction. This signifies that one extraction with butanol extracts as much as three extractions with ethyl acetate. Otherwise chloroform extracts 2 per cent of compound F and 3.8 per cent of compound E present in water.

The results obtained for the total 17-hydroxycorticosteroids in blood are higher than those already reported by other investigators (1, 5, 7). Although conjugated corticosteroids were discarded, the possibility exists that some splitting of the conjugates occurred during the evaporation step. No decomposition of the free corticosteroids was observed during the evaporation of the butanol.

In the procedure used in these investigations, cortisol and tetrahydrocortisone were consistently found in all blood samples in significant amounts. Cortisone and tetrahydrocortisol were present in much smaller amounts and could not be detected in some of the bloods.

TABLE III

Recovery of tetrahydrocortisone from Whatman N° 3 paper

Eluent	Applied µg	Recovered µg	Recovery %
Benzene	60	10	16
Chloroform	80	13	17
Alcohol-Ether	60	56	93
3:1	80	75	95

The ratio of cortisol to tetrahydrocortisone $\frac{F}{(THE)}$ appears to be relatively constant in peripheral blood (average value 0.62). In adrenal vein blood, Pincus (11) found this ratio to be 4.7 indicating much higher porportions of cortisol. When the ratio $\frac{THE}{F}$ is considered, the values averaged 1.5 in peripheral blood. In patients with Cushing's syndrome and in individuals receiving ACTH this ratio is decreased (12). This ratio was studied in three cases of Addisson's diseases; no cortisol was found but an spot corresponding to THE which ranged from 2 to 6 μg. of compound appeared (unpublished).

Summary

Data is presented refering to the solubility index and partition coefficient of different corticosteroids (THF, THE, F and E) using chloroform, butanol and ethyl acetate as solvent. Butanol extracts greater quantity of steroids from water solution than ethyl acetate and the latter a greater amount than chloroform.

Identification of corticosteroids by paper chromatography in blood extracts was carried out.

The total 17-hydroxycorticosteroids blood concentration ranged from 30 to 43.2 μg. per 100 ml. of blood. Cortisol and tetrahydrocortisone were consistently present in significant amounts, cortisone and tetrahydrocortisol, less regularly and in smaller amounts.

References

(1) Nelson, D. H. and Samuels, L. T.: *J. clin. Endocr.*, 1952, *12*, 515.
(2) Bush, I. E.: *Biochem. J.*, 1952, *50*, 370.
(3) Porter, C. C. and Silber, R. H.: *J. biol. Chem.*, 1950, *185*, 201.
(4) Levy, H. and Kushinsky, S.: *Rec. Progr. Horm. Res.*, 1954, *9*, 359.
(5) Bayliss, R. I. S. and Steinbeck, A. W.: *Memoirs of the Soc. for Endocrinol.*, 1953, *2*, 31.
(6) Reddy, W. J., Haydar, N. A., Laidlaw, J. C., Renold, A. E. and Thorn, G. W.: *J. clin. Endocrinol. & Metab.*, 1956, *27*, 380.
(7) Bush, I. E. and Sandberg, A. A.: *J. biol. Chem.*, 1953, *205*, 783.
(8) Zaffaroni, A. and Burton, R. B.: *J. biol. Chem.*, 1951, *193*, 749.
(9) del Campo de Hachen, C. P.: *Rev. argent. Endocrin. & Metab.*, 1956, *2*, 32.
(10) Zaffaroni, A.: *J. Amer. chem. Soc.*, 1950, *72*, 3828.
(11) Pingus, G.: *Ann. New York Acad. Sc.*, 1955, *27*, 380.
(12) del Campo de Hachen, C. P. and Solís, J.: *Rev. Soc. argent. Biol.*, 1956, *32*, 23.

We are very grateful to the kindness of Dr. Fidel E. Zelada, Head of the Blood Department of the Hospital Nacional del Centenario, Rosario, for supplying the blood that was used for extraction and to Dr. Eleanor H. Venning, Royal Victoria Hospital, Montreal, Canada, for her valuable discussion and personal correction of the manuscript and tables.

PROCEEDINGS OF THE ARGENTINE SOCIETY OF BIOLOGY

September 25, 1958

Method of permanent lateral cannulation in the aorta of the toad. M. C. Lico. *(Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires, Argentina).*

Different experimental studies in the toad (blood pressure, metabolism, blood composition) are made using the terminal aortic cannulation.

The usual method permits to use the animal only for 24 hours, as the tied aorta causes ischemic necrosis of the posterior limbs. This fact makes necessary the comparative measurements in an animal having a great individual variation and, therefore, raising statistical problems.

This paper describes a method of *permanent* lateral aortic cannulation without blocking the posterior limbs irrigation, thus allowing the animal to survive for 8 days or more and being his own control.

The aforementioned technique differs from the usual terminal cannulation, simply because ligature of the aorta takes only partially the aortic wall, thus performing a lateral cannulo-aortic anastomosis. This technical variation permits to maintain a sufficient circulation in the posterior limbs of the toad and the animal can remain cannulated during 8 days or more, withoud suffering damage.

Experimental modifications in the intrarenal pressure of dogs and rats. M. Cereijido. *(Instituto de Fisiología de la Facultad de Ciencias Médicas de Buenos Aires).*

Intrarenal pressure (IRP) was determined in rats and dogs before and after the ligature of the pedicle of the contralateral kidney.

The ligature of the renal pedicle produced in the contralateral kidney an increase of IRP which reached its highest value, (69 % above the control) 20 minutes after the ligature, and then decreased to a level slightly over the normal.

In rats, the administration of 3-5-3' triyodothyronine, which, has a potent renotrophic effect, also produced an increase of IRP; on the contrary, radiothyroidectomy produced a decrease of IRP.

Intestinal water absorption in the toad. J. Uranga. *(Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires, Argentina).*

Intestinal water absorption in the toad was not increased by dehydration or by administration of neurohypophyseal hormones.

It was also observed that the animals drink concentrated hypertonic solutions, but they do not drink water even being dehydrated.

Effect of purified gonadotrophins on the gonad of the "Bufo arenarum" Hensel after metamorphosis. M. H. BURGOS AND A. PISANO. *(Instituto de Histología y Embriología e Instituto de Biología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina).*

One month after metamorphosis, toads (*Bufo arenarum* Hensel) were injected with purified FSH and LH.

The gonads were in the female transitional period which preceeds the definite sex or they have just begun to differentiate into male or female (genotypes).

FSH masculinized all the specimens, including a genotypical female.

On the other hand, LH feminized all of them including a genotypical male.

These experiments show the bisexual nature of the gonads and reveal those purified gonadotrophins as powerful inductors of sex.

Observations on the gonad's growth of "Bufo arenarum" Hensel. A. PISANO AND N. PIZARRO. *(Instituto de Biología e Instituto de Histología y Embriología, Facultad de Ciencias Médicas, Universidad Nac. de Cuyo, Mendoza, Argentina).*

In the developing sexual gland of toads *Bufo arenarum* Hensel, three different zones are described: a cephalic (progonad), a medium (mesogonad) and a caudal (metagonad) one.

Histological characteristics show that gonad goes through a transient female phase before differentiating into testis or ovary.

This fact occurs 40 days, approximately, after metamorphosis.

A vasodilator substance present in purified angiotensin preparations. K. A. HALVORSEN, J. C. FASCIOLO, R. CALVO AND I. CHIONETTI. *(Cátedra de Fisiología, Facultad de Medicina, Mendoza, Argentina).*

It was demonstrated the presence of a vasodilator substance in a preparation of angiotensin, obtained by interaction of crude renin and angiotensinogen, purified by countercurrent method. Other preparations of angiotensin supplied by Dr. Paladini (PC2, PC3) were also rich in substance V.

This vasodilator substance is destroyed by enzymes in the blood, much more quickly than angiotensin itself.

The possibility exists of this substance being a polypeptide like bradykinin or kallidin or of other similar type.

Action of testosterone propionate on the hair growth of the white rat (1). A. B. HOUSSAY, R. NALLAR AND E. I. SAURER. *(Instituto de Biología y Medicina Experimental, Costa Rica 4185, and Centro de Endocrinología, Godoy Cruz 1221, Buenos Aires).*

1) Testosterone propionate, in adequate doses, prevents hair growth in normal, spayed, hypophysectomized or adrenalectomized rats.

2) The inhibitory effect on the hair follicle is much more intense in spayed than in normal rats, using the same doses.

3) Hypophysectomized rats are the most sensitive to the inhibitory effect of testosterone propionate.

---

(1) This paper is published *in extenso* in Acta Physiologica Latinoamericana.

5) Cortisone acetate reinforces the inhibitory action of testosterone propionate in adrenalectomized rats.

4) Adrenalectomized rats show the lowest sensitiveness to the effect of testosterone propionate, requiring larger doses to obtain an inhibitory effect.

Pressor and vasoconstrictor activity of Angiotensin I compared with Angiotensin II. K. A. HALVORSEN, J. C. FASCIOLO AND R. CALVO. *(Cátedra de Fisiología, Facultad de Medicina, Mendoza, Argentina).*

Isopressor doses of Angiotensin I and II (titrated on the blood pressure of dog or rat, by intravenous administration) have the same pressor effect on the anesthetized dog when injected by intraaortic or right intraventricular way, in the saphena, renal artery or perfused leg. Both substances produce the same vasoconstrictor effect in vascular preparations of *Bufo arenarum* Hensel, perfused with salin solution.

No proof has been found that Angiotensin I needs to be transformed in Angiotensin II to exert its role on the vessels.

Electron microscopy of pancreas, liver and salivary glands in guinea pigs treated with cobaltous chloride. P. E. LACY AND A. F. CARDEZA. *(Departamento de Patología, Washington University, St. Louis, Mo. EE. UU.).*

Electron microscopy of the pancreas, liver and parotid of the guinea pig treated with cobaltous chloride was studied. The daily dose used was 25 mg. of cobaltous chloride per Kg. of body weight in subcutaneous injection.

At 24 hours, partial degranulation, degenerating mitochondria and lipid droplets in the cytoplasm were observed in the acinar cells of the pancreas; at this time there were no changes in the ultra-structure of the alpha cells of the islets. The zymogen granules decreased progressively in number, and in the basal portion of the cells small granules surrounded by ergastoplasmic membranous sacs were observed. Hypertrophied and vacuolated mitochondria with disruption of their internal membranes were present. Later, giant mitochondria appeared in the acinar degranulated cells. Lipid droplets did not originate in the degenerating mitochondria.

The first changes in the ultra-structure of the alpha cells of the islets were observed after three days of treatment with cobaltous chloride and they consisted in vacuolization by dilatation of ergastoplasmic sacs and partial degranulation. The cytoplasm of vacuolated cells contained normal or dilated mitochondria, alpha cell granules, ergastoplasm, lipid droplets and portion of the Golgi complex. Total degranulation of the alpha cells was not present even in the most intense vacuolated forms.

When the treatment with cobaltous chloride was interrupted, these alterations in the acinar cells in the alpha cells of the islets were reversible.

These were no apparent changes in the ultra-structure of liver cells nor in the cells of the salivary glands.

Action of steroids on the ovulation of the toad "Bufo arenarum" Hensel. J. M. DE CORRAL. *(Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires, Argentina).*

The steroids used reinforce the action of subliminal doses of the *pars distalis* of the hypophysis of the toad, thus provoking ovulation. However, ovulation is not observed when injecting subliminal doses of *pars distalis* of hypophysis alone.

Ovulation is neither seen when steroids are given simultaneously to lower doses of hypophysis.

No ovulation is produced when steroids are given alone.

The reinforcing effect is exerted both *in vivo* and *in vitro* and is also observed in animals deprived of hypophysis, thyroid, adrenals and oviduct.

Preventive action of thyroidectomy with I^{131} on the diabetes of the rat. M. ALTIERI, P. BAZERQUE AND A. DENTI. *(Instituto de Fisiología, Facultad de Ciencias Médicas, Instituto de Biología y Medicina Experimental and Comisión Nacional de Energía Atómica, Buenos Aires).*

Iodothyroidectomy (I^{131}) prevents the apparition of diabetes in a high percentage (82 %) of white rats with large pancreatectomy (removal of 95 % of the pancreatic mass) compared with that observed in the controls (30 %) which were pancreatectomized at the same time and not treatd with radiactive iodine.

COMPAÑÍA IMPRESORA ARGENTINA S. A.
Alsina 2049 – Buenos Aires

REGLAMENTO DE PUBLICACION

Acta Physiologica Latinoamericana publica artículos originales inéditos o que hayan sido publicados previamente en forma parcial o completa en alguna revista local.

Los trabajos deben ser enviados al Jefe de Redacción del país de origen. Si no existiera Comité Editorial, deberán ser enviados a la Secretaría de Redacción de Acta Physiologica Latinoamericana, Obligado 2490, Buenos Aires, Argentina. La revista no se responsabiliza por los daños sufridos por el manuscrito o por su pérdida. Se recomienda a los autores conservar una copia completa de los trabajos que envíen por correo.

Preparación de los trabajos: Deberán ser escritos a máquina en papel tamaño carta, no transparente, a doble espacio y con amplio margen. Las ilustraciones deberán estar numeradas (fig. 1, fig. 2, etcétera), y llevar al pie una leyenda clara y concisa. Las fotografías hechas en papel brillante, nítidas. Los gráficos y diagramas, dibujados con tinta china sobre fondo blanco, listos para reproducir.

Se publicarán trabajos escritos en castellano, portugués, francés o inglés. Los que estén escritos en castellano o portugués deberán contener al final un resumen en inglés.

Las citas bibliográficas se harán en el texto mediante números [por ej.: algunos autores (1, 12) y en especial Jones (5)] o autores y año [por ej.: (Bre lauer, 1919)]. Al final del trabajo la bibliografía se ordenará alfabéticamente y con numeración progresiva, en el primer supuesto, y alfabéticamente en el segundo. Para las abreviaturas de las revistas, etc., se seguirán las recomendaciones del World List of Scientific Periodicals. La disposición de tales citas debe ajustarse a los ejemplos siguientes:

(1) Houssay, B. A., Lewis, J. T., Orías, O., Braun Menéndez, E., Hug, E., Foglia, V. G., Leloir, L. F.: *Fisiología Humana*, 3ª edición, El Ateneo, Buenos Aires 1954.

(2) Whittembury, G., Ramírez, M., Fernández, J., Monge, C.: *Acta physiol. latinoamer.*, 1955, *5*, 117.

De acuerdo con el carácter del artículo (artículo de conjunto o comunicación original) constará o no el título completo de los trabajos citados en la bibliografía.

Las medidas y símbolos deben expresarse de acuerdo con las recomendaciones de la Comisión de Símbolos, Unidades y Nomenclatura de la Unión Internacional de Física, aprobados en Amsterdam, en junio de 1948 (*Cienc. e Invest.*, 1949, *5*, 433).

SE EXPONEN A CONTINUACION ALGUNAS ABREVIATURAS COMUNES

	Castellano	*Inglés*
metro	m	m
centímetro	cm	cm
milímetro	mm	mm
micrón	μ	μ
milimicrón	$m\mu$	$m\mu$
Angström	Å	Å
microgramo	μg	μg
gama	γ	γ
hora	h	hr
minuto	m	Min
segundo	s	sec

	Castellano	*Inglés*
milisegundo	ms	msec
litro	l	l
centímetro cúbico	cm^3	cc
mililitro	ml	ml
kilogramo	kg	kg
gramo	g	gm
miligramo	mg	mg
miliequivalente	mEq	mEq
Curie	c	c
Milicurie	mc	mc
Microcurie	μC	μC
por ciento	%	%

Para evitar la confusión derivada de la notación decimal diferente según los países, se adopta el punto decimal y se suprime toda notación entre millares sustituyéndose por un espacio: 10 000 (no 10.000 ni 10,000) —0.90 (no 0,90).

Corrección de pruebas: Una de las pruebas de imprenta será remitida a los autores, quienes deberán devolverlas corregidas, dentro de los cuatro días subsiguientes a su recepción. Las modificaciones fundamentales en la corrección de las pruebas en desacuerdo con los originales, no serán tomadas en cuenta.

Pedido de apartados: Los autores que desen apartados podrán solicitarlos al devolver las pruebas. El costo correrá por su cuenta.

SUMARIO

E. Braun Menéndez. — J. T. Lewis 1

Nucleic Acid Content and Amylase Activity in the Pancreas of the Rat Following Adrenalectomy and Cortisone Administration. — A. Sesso and R. Migliorini 5

The Anabolic Action of Cortisone Upon the Exocrine Pancreas of the Suckling Rat. — A. Sesso and L. G. Oliverio 13

Endocrine Changes in Hemidecorticate Rats. — M. R. Covián, R. H. Migliorini and J. H. Tramezzani 24

Action of Testosterone Propionate Upon the Growth of Hair in Rats. — A. B. Houssay, R. Nallar and E. I. Saurer 35

Relationship Between Serum Creatinine, Endogenous Creatinine Clearance and Urinary Creatinine. — C. Monge C., M. Ramírez V., J. Fernández Ñ. and E. Horna C. 50

"Central" Blood Volume in Exercise in Normal Subjects. — A. J. Roncoroni, P. Aramendía, R. González and A. C. Taquini 55

Effect of Butyrate on Ventricular Fibrillation Induced by Lack of Metabolites on the Isolated Guinea Pig Heart. — M. Penna, A. Illanes, J. Ruiz and J. Mardones 66

Adrenocortical Hormone in Human Blood. — C. del Campo de Hachen .. 69

Proceedings of the Argentine Society of Biology 75